



PRODUCTION OF SYRINGYL LIGNIN IN GYMNOSPERMS

Field of the Invention

[0001] This application claims the benefit of U.S. Provisional Application No. 60/033,381, filed Dec. 16, 1996. The invention relates to the molecular modification of gymnosperms in order to cause the production of syringyl units during lignin biosynthesis and to production and propagation of gymnosperms containing syringyl lignin.

Background of the Invention

[0002] Lignin is a major part of the supportive structure of most woody plants including angiosperm and gymnosperm trees which in turn are the principal sources of fiber for making paper and cellulosic products. In order to liberate fibers from wood structure in a manner suitable for making many grades of paper, it is necessary to remove much of the lignin from the fiber/lignin network. Lignin is removed from wood chips by treatment of the chips in an alkaline solution at elevated temperatures and pressure in an initial step of papermaking processes. The rate of removal of lignin from wood of different tree species varies depending upon lignin structure. Three different lignin structures have been identified in trees: p-hydroxyphenyl, guaiacyl and syringyl, which are illustrated in FIG. 1.

[0003] Angiosperm species, such as *Liquidambar styraciflua* L. [sweetgum], have lignin composed of a mixture of guaiacyl and syringyl monomer units. In contrast, gymnosperm species such as *Pinus taeda* L. [loblolly pine] have lignin which is devoid of syringyl monomer units. Generally speaking, the rate of delignification in a pulping process is directly proportional to the amount of syringyl lignin present in the wood. The higher delignification rates associated with species having a greater proportion of syringyl lignin result in more efficient pulp mill operations since the mills make better use of energy and capital investment and the environmental impact is lessened due to a decrease in chemicals used for delignification.

[0004] It is therefore an object of the invention to provide gymnosperm species which are easier to delignify in pulping processes.

[0005] Another object of the invention is to provide gymnosperm species such as loblolly pine which contain syringyl lignin.

[0006] An additional object of the invention is to provide a method for modifying genes involved in ~~lignin~~ lignin biosynthesis in gymnosperm species so that production of syringyl lignin is increased while production of guaiacyl lignin is suppressed.

[0007] Still another object of the invention is to produce whole gymnosperm plants containing genes which increase production of syringyl lignin and repress production of guaiacyl lignin.

[0008] Yet another object of the invention is to identify, isolate and/or clone those genes in angiosperms responsible for production of syringyl lignin.

[0009] A further object of the invention is to provide, in gymnosperms, genes which produce syringyl lignin.

[0010] Another object of the invention is to provide a method for making an expression cassette insertable into a gymnosperm cell for the purpose of inducing formation of syringyl lignin in a gymnosperm plant derived from the cell.

Definitions

[0011] The term "promoter" refers to a DNA sequence in the 5' flanking region of a given gene which is involved in recognition and binding of RNA polymerase and other transcriptional proteins and is required to initiate DNA transcription in cells.

[0012] The term "constitutive promoter" refers to a promoter which activates transcription of a desired gene, and is commonly used in creation of an expression cassette designed for preliminary experiments relative to testing of gene function. An example of a constitutive promoter is 35S CaMV, available from Clontech.

[0013] The term "expression cassette" refers to a double stranded DNA sequence which contains both promoters and genes such that expression of a given gene is achieved upon insertion of the expression cassette into a plant cell.

[0014] The term "plant" includes whole plants and portions of plants, including plant organs (e.g. roots, stems, leaves, etc.)

[0015] The term "angiosperm" refers to plants which produce seeds encased in an ovary. A specific example of an angiosperm is *Liquidambar styraciflua* (L.) [sweetgum]. The angiosperm sweetgum produces syringyl lignin.

[0016] The term "gymnosperm" refers to plants which produce naked seeds, that is, seeds which are not encased in an ovary. A specific example of a gymnosperm is *Pinus taeda* (L.) [loblolly pine]. The gymnosperm loblolly pine does not produce syringyl lignin.

Summary of the Invention

[0017] With regard to the above and other objects, the invention provides a method for inducing production of syringyl lignin in gymnosperms and to gymnosperms which contain syringyl lignin for improved delignification in the production of pulp for papermaking and other applications. In accordance with one of its aspects, the invention involves cloning an angiosperm DNA sequence which codes for enzymes involved in production of syringyl lignin monomer units, fusing the angiosperm DNA sequence to a lignin promoter region to form an expression cassette, and inserting the expression cassette into a gymnosperm genome.

[0018] Enzymes required for production of syringyl lignin in an angiosperm are obtained by deducing an amino acid sequence of the enzyme, extrapolating an mRNA sequence from the amino acid sequence, constructing a probe for the corresponding DNA sequence and cloning the DNA sequence which codes for the desired enzyme. A promoter region specific to a gymnosperm lignin biosynthesis gene is identified by constructing a probe for a

gymnosperm lignin biosynthesis gene, sequencing the 5' flanking region of the DNA which encodes the gymnosperm lignin biosynthesis gene to locate a promoter sequence, and then cloning that sequence.

[0019] An expression cassette is constructed by fusing the angiosperm syringyl lignin DNA sequence to the gymnosperm promoter DNA sequence. Alternatively, the angiosperm syringyl lignin DNA is fused to a constitutive promoter to form an expression cassette. The expression cassette is inserted into the gymnosperm genome to transform the gymnosperm genome. Cells containing the transformed genome are selected and used to produce a transformed gymnosperm plant containing syringyl lignin.

[0020] In accordance with the invention, the angiosperm gene sequences bi-OMT, 4CL, FA5HP450-1 and FA5HP-450-2 have been determined and isolated as associated with production of syringyl lignin in sweetgum and lignin promoter regions for the gymnosperm loblolly pine have been determined to be the 5' flanking regions for the 4CL1B, 4CL3B and PAL gymnosperm lignin genes. Expression cassettes containing sequences of selected genes from sweetgum have been inserted into loblolly pine embryogenic cells and presence of sweetgum genes associated with production of syringyl lignin has been confirmed in daughter cells of the resulting loblolly pine embryogenic cells.

[0021] The invention therefore enables production of gymnosperms such as loblolly pine containing genes which code for production of syringyl lignin, to thereby produce in such species syringyl lignin in the wood structure for enhanced pulpability.

Brief Description of the Drawings

[0022] The above and other aspects of the invention will now be further described in the following detailed specification considered in conjunction with the following drawings in which:

[0023] FIG. 1 illustrates a generalized pathway for lignin synthesis; and

[0024] ~~FIG. 2 illustrates~~ FIGS. 2A-2E illustrate a bifunctional-O-methyl transferase (bi-OMT) gene sequence involved in the production of syringyl lignin in an angiosperm (SEQ ID 35 coding SEQ ID 6);

[0025] ~~FIG. 3 illustrates~~ FIGS. 3A-3G illustrate a 4-coumarate CoA ligase (4CL) gene sequence involved in the production of syringyl lignin in an angiosperm (SEQ ID 47 coding SEQ ID 8);

[0026] FIG. 4 illustrates a ferulic acid-5-hydroxylase (~~FA5HP450~~-1) gene sequence involved in the production of syringyl lignin in an angiosperm (SEQ ID 1 coding SEQ ID 2);

[0027] FIG. 5 illustrates a ferulic acid-5-hydroxylase (~~FA5HP450~~-2) gene sequence involved in the production of syringyl lignin in an angiosperm (SEQ ID 23 coding SEQ ID 4);

[0028] FIG. 6 illustrates nucleotide sequences of the 5' flanking region of the loblolly pine 4CL1B gene showing the location of regulatory elements for lignin biosynthesis (SEQ ID 610);

[0029] ~~FIG. 7 illustrates~~ FIGS 7A-7B illustrate nucleotide sequences of the 5' flanking region of the loblolly pine 4CL3B gene showing the location of regulatory elements for lignin biosynthesis (SEQ ID 711);

[0030] ~~FIG. 8 illustrates~~ FIGS. 8A-8B illustrate nucleotide sequences of the 5' flanking region of loblolly pine PAL gene showing the location of regulatory elements for lignin biosynthesis (SEQ ID 59);

[0031] FIG. 9 illustrates a PCR confirmation of the sweetgum ~~FA5HP450~~-1 gene sequence in transgenic loblolly pine cells; and

Detailed Description of the Invention

[0032] In accordance with the invention, a method is provided for modifying a gymnosperm genome, such as the genome of a loblolly pine, so that syringyl lignin will be

produced in the resulting plant, thereby enabling cellulosic fibers of the same to be more easily separated from lignin in a pulping process. In general, this is accomplished by fusing one or more angiosperm DNA sequences (referred to at times herein as the "ASL DNA sequences") which are involved in production of syringyl lignin to a gymnosperm lignin promoter region (referred to at times herein as the "GL promoter region") specific to genes involved in gymnosperm lignin biosynthesis to form a gymnosperm syringyl lignin expression cassette (referred to at times herein as the "GSL expression cassette").

Alternatively, the one or more ASL DNA sequences are fused to one or more constitutive promoters to form a GSL expression cassette.

[0033] The GSL expression cassette preferably also includes selectable marker genes which enable transformed cells to be differentiated from untransformed cells. The GSL expression cassette containing selectable marker genes is inserted into the gymnosperm genome and transformed cells are identified and selected, from which whole gymnosperm plants may be produced which exhibit production of syringyl lignin.

[0034] To suppress production of less preferred forms of lignin in gymnosperms, such as guaiacyl lignin, genes from the gymnosperm associated with production of these less preferred forms of lignin are identified, isolated and the DNA sequence coding for anti-sense mRNA (referred to at times herein as the "GL anti-sense sequence") for these genes is produced. The DNA sequence coding for anti-sense mRNA is then incorporated into the gymnosperm genome, which when expressed bind to the less preferred guaiacyl gymnosperm lignin mRNA, inactivating it.

[0035] Further features of these and various other steps and procedures associated with practice of the invention will now be described in more detail beginning with identification and isolation of ASL DNA sequences of interest for use in inducing production of syringyl lignin in a gymnosperm.

I. Determination Of DNA Sequence For Genes Associated With Production Of Syringyl Lignin

[0036] The general biosynthetic pathway for production of lignin has been postulated as shown in FIG. 1. From FIG. 1, it can be seen that the genes CCL, OMT and F5H (which is from the class of P450 genes) may play key roles in production of syringyl lignin in some plant species, but their specific contributions and mechanisms remain to be positively established. It is suspected that the CCL, OMT and F5H genes may have specific equivalents in a specific angiosperm, such as sweetgum. Accordingly, one aim of the present invention is to identify, sequence and clone specific genes of interest from an angiosperm such as sweetgum which are involved in production of syringyl lignin and to then introduce those genes into the genome of a gymnosperm, such as loblolly pine, to induce production of syringyl lignin.

[0037] Genes of interest may be identified in various ways, depending on how much information about the gene is already known. Genes believed to be associated with production of syringyl lignin have already been sequenced from a few angiosperm species, viz, CCL and OMT.

[0038] DNA sequences of the various CCL and OMT genes are compared to each other to determine if there are conserved regions. Once the conserved regions of the DNA sequences are identified, oligo-dT primers homologous to the conserved sequences are synthesized. Reverse transcription of the DNA-free total RNA which was purified from sweetgum xylem tissue, followed by double PCR using gene-specific primers, enables production of probes for the CCL and OMT genes.

[0039] A sweetgum cDNA library is constructed in a host, such as lambda ZAPII, available from Stratagene, of LaJolla, Calif., using poly(A)+RNA isolated from sweetgum xylem, according to the methods described by Bugos et al. (1995 Biotechniques 19:734-737). The above mentioned probes are used to assay the sweetgum cDNA library to locate cDNA which

codes for enzymes involved in production of syringyl lignin. Once a syringyl lignin sequence is located, it is then cloned and sequenced according to known methods which are familiar to those of ordinary skill.

[0040] In accordance with the invention, two sweetgum syringyl lignin genes have been determined using the above-described technique. These genes have been designated 4CL and bi-OMT. The sequence obtained for the sweetgum syringyl lignin gene, designated bi-OMT, is illustrated in FIG. 2 (SEQ ID ~~35~~ and 6). The sequence obtained for the sweetgum syringyl lignin gene, designated 4CL, is illustrated in FIG. 3 (~~SEQ ID 4~~ SEQ ID 7 and 8).

[0041] An alternative procedure was employed to identify the F5H equivalent genes in sweetgum. Because the DNA sequences for similar P450 genes from other plant species were known, probes for the P450 genes were designed based on the conserved regions found by comparing the known sequences for similar P450 genes. The known P450 sequences used for comparison include all plant P450 genes in the GenBank database. Primers were designed based on two highly conserved regions which are common to all known plant P450 genes. The primers were then used in a PCR reaction with the sweetgum cDNA library as a template. Once P450-like fragments were located, they were amplified using standard PCR techniques, cloned into a pBluescript vector available from Clontech of Palo Alto, Calif. and transformed into a DH5.alpha. E. coli strain available from Gibco BRL of Gaithersburg, Md.

[0042] After *E. coli* colonies were tested in order to determine that they contained the P450-like DNA fragments, the fragments were sequenced. Several P450-like sequences were located in sweetgum using the above described technique. One P450-like sequence was sufficiently different from other known P450 sequences to indicate that it represented a new P450 gene family. This potentially new P450 cDNA fragment was used as a probe to screen a full length clone from the sweetgum xylem library. ~~This~~ These putative hydroxylase ~~clone~~ was designated FA5HP450-1 clones were designated P450-1 and P450-2. The sequence

obtained for ~~FA5H1~~ P450-1 and P450-2 are ~~is~~-illustrated in FIG. 4 (SEQ ID ~~41~~ and 2) and FIG. 5 (SEQ ID 3 and 4).

II. Identification Of GL Gene Promoter Regions

[0043] In order to locate gymnosperm lignin promoter regions, probes are developed to locate lignin genes. After the-gymnosperm lignin gene is located, the portion of DNA upstream from the gene is sequenced, preferably using the GenomeWalker Kit, available from Clontech. The portion of DNA upstream from the lignin gene will generally contain the gymnosperm lignin promoter region.

[0044] Gymnosperm genes of interest include CCL-like genes and PAL-like genes, which are beleived to be involved in the production of lignin in gymnosperms. Preferred probe sequences are developed based on previously sequenced genes, which are available from the gene bank. The preferred gene bank accession numbers for the CCL-like genes include U39404 and U39405. A preferred gene bank accession number for a PAL-like gene is U39792. Probes for such genes are constructed according to methods familiar to those of ordinary skill in the art. A genomic DNA library is constructed and DNA fragments which code for gymnosperm lignin genes are then identified using the above mentioned probes. A preferred DNA library is obtained from the gymnosperm, *Pinus taeda* (L.) [Loblolly Pine], and a preferred host of the genomic library is Lambda DashII, available from Stratagene of LaJolla, Calif.

[0045] Once the DNA fragments which code for the gymnosperm lignin genes are located, the genomic region upstream from the gymnosperm lignin gene (the 5' flanking region) was identified. This region contains the GL promoter. Three promoter regions were located from gymnosperm lignin biosynthesis genes. The first is the 5' flanking region of the loblolly pine 4CL1B gene, shown in FIG. 6 (SEQ ID 610). The second is the 5' flanking region of the

loblolly pine gene 4CL3B, shown in FIG. 7 (SEQ ID 711). The third is the 5' flanking region of the loblolly pine gene PAL, shown in FIG. 8 (SEQ ID 59).

III. Fusing The GL Promoter Region To The ASL DNA Sequence

[0046] The next step of the process is to fuse the GL promoter region to the ASL DNA sequence to make a GSL expression cassette for insertion into the genome of a gymnosperm. This may be accomplished by standard techniques. In a preferred method, the GL promoter region is first cloned into a suitable vector. Preferred vectors are pGEM7Z, available from Promega, Madison, Wis. and SK available from Stratagene, of LaJolla, Calif. After the promoter sequence is cloned into the vector, it is then released with suitable restriction enzymes. The ASL DNA sequence is released with the same restriction enzyme(s) and purified.

[0047] The GL promoter region sequence and the ASL DNA sequence are then ligated such as with T4 DNA ligase, available from Promega, to form the GSL expression cassette. Fusion of the GL and ASL DNA sequence is confirmed by restriction enzyme digestion and DNA sequencing. After confirmation of GL promoter-ASL DNA fusion, the GSL expression cassette is released from the original vector with suitable restriction enzymes and used in construction of vectors for plant transformation.

IV. Fusing The ASL DNA Sequence to a Constitutive Promoter Region

[0048] In an alternative embodiment, a standard constitutive promoter may be fused with the ASL DNA sequence to make a GSL expression cassette. For example, a standard constitutive promoter may be fused with FA5HP450-1 to form an expression cassette for insertion of FA5HP450-1 sequences into a gymnosperm genome. In addition, a standard constitutive promoter may be fused with FA5HP450-2 to form an expression cassette for insertion of FA5HP450-2 into a gymnosperm genome. A constitutive promoter for use in the invention is the double 35S promoter, available from Clontech.

[0049] In the preferred practice of the invention using constitutive promoters, a suitable vector such as pBipBI221, is digested XbaI and HindIII to release the 35S promoter. At the same time the vector pHygro, available from International Paper, was digested by XbaI and HindIII to release the double 35S promoter. The double 35S promoter was ligated to the previously digested pBipBI221 vector to produce a new pBipBI221 with the double 35S promoter. This new pBipBI221 was digested with SacI and SmaI, to release the GUS fragment. The vector is next treated with T4 DNA polymerase to produce blunt ends and the vector is self-ligated. This vector is then further digested with BamHI and XbaI, available from Promega. After the pBipBI221 vector containing the constitutive promoter region has been prepared, lignin gene sequences are prepared for insertion into the pBipBI221 vector.

[0050] The coding regions of sweetgum FA5HP450-1 or FA5HP450-2 are amplified by PCR using primer with restriction sites incorporated in the 5' and 3' ends. In one example, an XbaI site was incorporated at the 5' end and a ~~BamHI~~-BamHI site was incorporated at the 3' end of the sweetgum FA5HP450-1 or FA5HP450-2 genes. After PCR, the FA5HP450-1 and FA5HP450-2 genes were separately cloned into a TA vector available from Invitrogen. The TA vectors containing the FA5HP450-1 and FA5HP450-2 genes, respectively, were digested by XbaI and BamHI to release the FA5HP450-1 or FA5HP450-2 sequences.

[0051] The p35SS vector, described above, and the isolated sweetgum FA5HP450-1 or FA5HP450-2 fragments were then ligated to make GLS expression cassettes containing the ~~constitutive~~-constitutive promoter.

V. Inserting the Expression Cassette into the Gymnosperm Genome

[0052] There are a number of methods by which the GLS expression cassette may be inserted into a target gymnosperm cell. One method of inserting the expression cassette into the gymnosperm is by micro-projectile bombardment of gymnosperm cells. For example, embryogenic tissue cultures of loblolly pine may be initiated from immature zygotic

embryos. Tissue is maintained in an undifferentiated state on semi-solid proliferation medium. For transformation, embryogenic tissue is suspended in liquid proliferation medium. Cells are then sieved through, a preferably 40 mesh screen, to separate small, densely cytoplasmic cells from large vacuolar cells.

[0053] After separation, a portion of the liquid cell suspension fraction is vacuum deposited onto filter paper and placed on semi-solid proliferation medium. The prepared gymnosperm target cells are then grown for several days on filter paper discs in a petri dish.

[0054] A 1:1 mixture of plasmid DNA containing the selectable marker expression cassette and plasmid DNA containing the FASHP450-1 expression cassette may be precipitated with gold to form microprojectiles. The microprojectiles are rinsed in absolute ethanol and aliquots are dried onto a suitable macrocarrier such as the macrocarrier available from BioRad in Hercules, Calif.

[0055] Prior to bombardment, embryogenic tissue is preferably desiccated under a sterile laminar-flow hood. The desiccated tissue is transferred to semi-solid proliferation medium. The prepared microprojectiles are accelerated from the macrocarrier into the desiccated target cells using a suitable apparatus such as a BioRad PDS-1000/HE particle gun. In a preferred method, each plate is bombarded once, rotated 180 degrees, and bombarded a second time. Preferred bombardment parameters are 1350 psi rupture disc pressure, 6 mm distance from the rupture disc to macrocarrier (gap distance), 1 cm macrocarrier travel distance, and 10 cm distance from macrocarrier stopping screen to culture plate (microcarrier travel distance). Tissue is then transferred to semi-solid proliferation medium containing a selection agent, such as hygromycin B, for two days after bombardment.

[0056] Other methods of inserting the GSL expression cassette include use of silicon carbide whiskers, transformed protoplasts, *Agrobacterium* vectors and electroporation.

VI. Identifying Transformed Cells

[0057] In general, insertion of the GSL expression cassette will typically be carried out in a mass of cells and it will be necessary to determine which cells harbor the recombinant DNA molecule containing the GSL expression cassette. Transformed cells are first identified by their ability to grow vigorously on a medium containing an antibiotic which is toxic to non-transformed cells. Preferred antibiotics are kanamycin and hygromycin B. Cells which grow vigorously on antibiotic containing medium are further tested for presence of either portions of the plasmid vector, the syringyl lignin genes in the GSL expression cassette; e.g. the angiosperm bi-OMT, 4CL, ~~FA5HP450~~-1 or ~~FA5HP450~~-2 gene, or by testing for presence of other fragments in the GSL expression cassette. Specific methods which can be used to test for presence of portions of the GSL expression cassette include Southern blotting with a labeled complementary probe or PCR amplification with specific complementary primers. In yet another approach, an expressed syringyl lignin enzyme can be detected by Western blotting with a specific antibody, or by assaying for a functional property such as the appearance of functional enzymatic activity.

VII. Production of a Gymnosperm Plant from the Transformed Gymnosperm Cell

[0058] Once transformed embryogenic cells of the gymnosperm have been identified, isolated and multiplied, they may be grown into plants. It is expected that all plants resulting from transformed cells will contain the GSL expression cassette in all their cells, and that wood in the secondary growth stage of the mature plant will be characterized by the presence of syringyl lignin.

[0059] Transgenic embryogenic cells are allowed to replicate and develop into a somatic embryo, which are then converted into a somatic seedling.

VIII. Identification, Production and Insertion of a GL mRNA Anti-Sense Sequence

[0060] In addition to adding ASL DNA sequences, anti-sense sequences may be incorporated into a gymnosperm genome, via GSL expression cassettes, in order to suppress

formation of the less preferred native gymnosperm lignin. To this end, the gymnosperm lignin gene is first located and sequenced in order to determine its nucleotide sequence. Methods for locating and sequencing amino acids which have been previously discussed may be employed. For example, if the gymnosperm lignin gene has already been purified, standard sequencing methods may be employed to determine the DNA nucleic acid sequence.

[0061] If the gymnosperm lignin gene has not been purified and functionally similar DNA or mRNA sequences from similar species are known, those sequences may be compared to identify highly conserved regions and this information used as a basis for the construction of a probe. A gymnosperm cDNA or genomic library can be probed with the above mentioned sequences to locate the gymnosperm lignin cDNA or genomic DNA. Once the gymnosperm lignin DNA is located, it may be sequenced using standard sequencing methods.

[0062] After the DNA sequence has been obtained for a gymnosperm lignin sequence, the complementary anti-sense strand is constructed and incorporated into an expression cassette. For example, the GL mRNA anti-sense sequence may be fused to a promoter region to form an expression cassette as described above. In a preferred method, the GL mRNA anti-sense sequence is incorporated into the previously discussed GSL expression cassette which is inserted into the gymnosperm genome as described above.

IX. Inclusion of Cytochrome P450 Reductase (CPR) to Enhance Biosynthesis Of Syringyl Lignin in Gymnosperms

[0063] In the absence of external cofactors such as NADPH (an electron donor in reductive biosyntheses), certain angiosperm lignin genes such as the ~~FA5~~HP450 genes may remain inactive or not achieve full or desired activity after insertion into the genome of a gymnosperm. Inactivity or insufficient activity can be determined by testing the resulting plant which contains the ~~FA5~~HP450 genes for the presence of syringyl lignin in secondary growth. It is known that cytochrome P450 reductase (CPR) may be involved in promoting certain reductive biochemical reactions, and may activate the desired expression of genes in

many plants. Accordingly, if it is desired to enhance the expression of the angiosperm syringyl lignin genes in the gymnosperm, CPR may be inserted in the gymnosperm genome. In order to express CPR, the DNA sequence of the enzyme is ligated to a constitutive promoter or, for a specific species such as loblolly pine, xylem-specific lignin promoters such as PAL, 4CL1B or 4CL3B to form an expression cassette. The expression cassette may then be inserted into the gymnosperm genome by various methods as described above.

X. Examples

[0064] The following non-limiting examples illustrate further aspects of the invention. In these examples, the angiosperm is *Liquidambar styraciflua* (L.) [sweetgum] and the gymnosperm is *Pinus taeda* (L.) [loblolly pine]. The nomenclature for the genes referred to in the examples is as follows:

Genes	Biochemical Name
4CL (angiosperm)	4-coumarate CoA ligase
bi-OMT (angiosperm)	bifunctional-O-methyl transferase
FA5HP450-1 (angiosperm)	Cytochrome P450 ferulic acid 5-hydroxylase
FA5HP450-2 (angiosperm)	Cytochrome P450 ferulic acid 5-hydroxylase
PAL (gymnosperm)	phenylalanine ammonia-lyase
4CL1B (gymnosperm)	4-coumarate CoA ligase
4CL3B (gymnosperm)	4-coumarate CoA ligase

Example 1 - Isolating and Sequencing bi-OMT and 4CL Genes from an Angiosperm

[0065] A cDNA library for Sweetgum was constructed in Lambda ZAPII, available from Stratagene, of LaJolla, Calif., using poly(A)+RNA isolated from Sweetgum xylem tissue.

Probes for bi-OMT and 4CL were obtained through reverse transcription of their mRNAs and

followed by double PCR using gene-specific primers which were designed based on the OMT and CCL cDNA sequences obtained from similar genes cloned from other species.

[0066] Three primers were used for amplifying OMT fragments. One was an oligo-dT primer. One was a 5'-bi-OMT, (which was cloned through used to clone gene fragments through modified differential display technique, as described below in Example 2) and the other two were degenerate primers, which were based on the conserved sequences of all known OMTs. The two degenerate primers were derived based on the following amino acid sequences:

5'-Gly Gly Met Ala Thr Tyr Cys Cys Ala Thr Thr Tyr Ala Ala Cys Ala Ala Gly Gly Cys-3' (primer #22) (SEQ ID 12) and

3'-Ala Ala Ala Gly Ala Gly Ala Gly Asn Ala Cys Asn Asn Ala Asn Asn Ala Asn Gly Ala-5' (primer #23) (SEQ ID 13).

[0067] A 900 bp PCR product was produced when oligo-dT primer and primer #22 were used, and a 550 bp fragment was produced when primer numbers 22 and 23 were used.

[0068] Three primers were used for amplifying CCL fragments. They were derived from the following amino acid sequences:

5'-Thr Thr Gly Gly Ala Thr Cys Cys Gly Gly Ile Ala Cys Ile Ala Cys Ile Gly Gly Ile Tyr Thr Ile Cys Cys Ile Ala Ala Arg Gly Gly-3' (primer R1S) (SEQ ID 14)

5'-Thr Thr Gly Gly Ala Thr Cys Cys Gly Thr Ile Gly Thr Ile Gly Cys Ile Cys Ala Arg Cys Ala Arg Gly Thr Ile Gly Ala Tyr Gly Gly-3' (primer H1S) (SEQ ID 15) and

3'-Cys Cys Ile Cys Thr Tyr Thr Ala Asp Ala Cys Arg Thr Ala Asp Gly Cys Ile Cys Cys Ala Gly Cys Thr Gly Thr Ala-5' (primer R2A) (SEQ ID 16)

[0069] R1S and H1S were both sense primers. Primer R2A was an anti-sense primer. A 650 bp fragment was produced if R1S and R2A primers were used and a 550 bp fragment was

produced when primers H1S and R2A were used. The sequence of these three primers were derived from conserved sequences for plant CCLs.

[0070] The reverse transcription-double PCR cloning technique used for these examples consisted of adding 10 ~~µg~~ of DNA-free total RNA in 25 ~~µl~~ DEPC-treated water to a microfuge tube. Next, the following solutions were added:

- a. 5x Reverse transcript buffer 8.0 µl,
- b. 0.1 ~~µM~~ ~~DDT~~ MDTT 4.0 µl
- c. 10 mM dNTP 2.0 µl
- d. 100 µM oligo-dT primers 8.0 µl
- e. Rnasin 2.0 µl
- f. Superscript II 1.0 µl

[0071] After mixing, the tube was incubated at a temperature of 42° C. for one (1) hour, followed by incubation at 70° C. for fifteen (15) minutes. Forty (40) µl of 1N NaOH was added and the tube was further incubated at 68° C. for twenty (20) minutes. After the incubation periods, 80 µl of 1N HCl was added to the reaction mixture. At the same time, 17 µl NaOAc, 5 µl glycogen and 768 µl of 100% ethanol were added and the reaction mixture was maintained at -80° C. for 15 minutes in order to precipitate the cDNA. The precipitated cDNA was centrifuged at high speed at 4° C. for 15 minutes. The resulting pellet was washed with 70% ethanol and then dried at room temperature, and then was dissolved in 20 µl of water.

[0072] The foregoing procedure produced purified cDNA which was used as a template to carry out first round PCR using primers #22 and oligo-dT for cloning OMT cDNA and primer R1S and R2A for cloning 4CL cDNA. For the first round PCR, a master mix of 50 µl for each reaction was prepared. Each 50 µl mixture contained:

- a. 10x buffer 5 µl

- b. 25 mM MgCl₂ 5 µl
- c. 100 µM sense primer 1 µl (primer #22 for OMT and primer R1S for CCL).
- d. 100 µl anti-sense primer 1 µl (oligo-dT primer for OMT and R2A for CCL).
- e. 10 mM dNTP 1 µl
- f. Taq. DNA polymerase 0.5 µl

[0073] Of this master mix, 48 µl was added into a PCR tube containing 2 µl of cDNA for PCR. The tube was heated to 95° C. for 45 seconds, 52° C. for one minute and 72° C. for two minutes. This temperature cycle was repeated for 40 cycles and the mixture was then held at 72° C. for 10 minutes.

[0074] The cDNA fragments obtained from the first round of PCR were used as templates to perform the second round of PCR using primers 22 and 23 for cloning bi-OMT cDNA and primer H1S and R2A for cloning 4CL cDNA. The second round of PCR conditions were the same as the first round.

[0075] The desired cDNA fragment was then ~~sub-cloned~~ subcloned and sequenced. After the second round of PCR, the product with the predicted size was excised from the gel and ligated into a pUC19 vector, available from Clonotech, of Palo Alto, Calif., and then transformed into DH5.alpha., an E. coli strain, available from Gibco BRL, of Gaithersburg, Md. After the inserts had been checked for correct size, the colonies were isolated and plasmids were sequenced using a Sequenase kit available from USB, of Cleveland, Ohio. The sequences are shown in FIG. 2 (SEQ ID ~~35~~ and 6) and FIG. 3 (~~SEQ ID 4~~ SEQ ID 7 and 8).

Example 2 - Alternative Isolation Method of Angiosperm bi-OMT Gene

[0076] As previously mentioned, one bi-OMT clone was produced via modified differential display technique. This method is another type of reverse transcription-PCR, in which DNA-free total RNA was reverse transcribed using oligo-dT primers with a single base pair anchor

to form cDNA. The oligo-dT primers used for reverse transcription of mRNA to synthesize cDNA were:

4 T11A: TTTTTTTTTTTTTTA, [\(SEQ ID 17\)](#)

T11C: TTTTTTTTTTTTTTC, [\(SEQ ID 18\)](#) and

T11G: TTTTTTTTTTTTTTG, [\(SEQ ID 19\)](#)

[0077] These cDNAs were then used as templates for radioactive PCR which was conducted in the presence of the same oligo-dT primers as listed above, a bi-OMT gene-specific primer and 35S-dATP. The OMT gene-specific primer was derived from the following amino acid sequence:

5'-Cys Cys Asn Gly Gly Asn Gly Gly Ser Ala Arg Gly Ala-3'. [\(SE ID 20\)](#)

[0078] The following PCR reaction solutions were combined in a microfuge tube:

- a. H₂O 9.2 µl,
- b. Taq Buffer 2.0 µl
- c. dNTP (25 µM) 1.6 µl
- d. Primers (5 µM) 2 µl, for each primer
- e. ³⁵S-dATP 1 µl
- f. Taq. pol. 0.2 µl
- g. cDNA 2.0 µl.

[0079] The tube was heated to a temperature of 94° C. and held for 45 seconds, then at 37° C. for 2 minutes and then 72° C. for 45 seconds for forty cycles, followed by a final reaction at 72° C. for 5 minutes.

[0080] The amplified products were fractionated on a denaturing polyacrylamide sequencing gel and autoradiography was used to identify and excise the fragments with a predicted size. The designed OMT gene-specific primer had a sequence conserved in a region

toward the 3'-end of the OMT cDNA sequence. This primer, together with oligo-dT, was amplified into a OMT cDNA fragment of about 300 bp.

[0081] Three oligo-dTs with a single base pair of A, C or G, respectively, were used to pair with the OMT gene-specific primer. Eight potential OMT cDNA fragments with predicted sizes of about 300 bp were excised from the gels after several independent PCR rounds using different combinations of oligo-dT and OMT gene-specific oligo-nucleotides as primers.

[0082] The OMT cDNA fragments were then re-amplified. A Southern blot analysis was performed for the resulting cDNAs using a 360 base-pair, ³²P radio-isotope labeled, aspen OMT cDNA 3'-end fragment as a probe to identify the cDNA fragments having a strong hybridization signal, under low stringency conditions. Eight fragments were identified. Out of these eight cDNA fragments, three were selected based on their high hybridization signal for sub-cloning and sequencing. One clone, LsOMT3'-1, (where the "Ls" prefix indicates that the clone was derived from the Liquidambar styraciflua (L.) genome) was confirmed to encode bi-OMT based on its high homology to other lignin-specific plant OMTs at both nucleotide and amino acid sequence levels.

[0083] A cDNA library was constructed in Lambda ZAP II, available from Stratagene, of LaJolla, Calif., using 5 mg poly(A)+RNA isolated from sweetgum xylem tissue. The primary library consisting of approximately 0.7×10^6 independent recombinants was amplified and approximately 10^5 plaque-forming-units (pfu) were screened using a homologous 550 base-pair probe. The hybridized filter was washed at high stringency (0.25xSSC, 0.1% SDS, 65° C.) conditions. The colony containing the bi-OMT fragment identified by the probe was eluted and the bi-OMT fragment was produced. The sequence as illustrated in FIG. 2 (SEQ ID 35 and 6) was obtained.

Example 3 - Isolating and Producing the DNA which Codes for the Angiosperm FA5HP450-1 Gene

[0084] In order to find putative FA5HP450 cDNA fragments as probes for cDNA library screening, a highly degenerated sense primer based on the amino acid sequence of 5'-Glu, Glu, Phe, Arg, Pro, Glu, Arg-3' was designed based on the conserved regions found in some plant P450 proteins. This conserved domain was located upstream of another highly conserved region in P450 proteins, which had an amino acid sequence of 5'-Phe Gly Xaa Gly Xaa Xaa Cys Xaa Gly-3' (SEQ ID 21). This primer was synthesized with the incorporation of an XboI restriction site to give a 26-base-pair oligomer with a nucleotide sequence of 5' ATG TGC AGT TTT TTT TTT TTT TIT TT-3' (SEQ ID 22).

[0085] This primer and the oligo-dT-XhoI primer were then used to perform PCR reactions with the sweetgum cDNA library as a template. The cDNA library was constructed in Lambda ZAPII, available from Stratagene, of LaJolla, Calif., using poly(a)+RNA isolated from Sweetgum xylem tissue. Amplified fragments of 300 to 600 bp were obtained. Because the designed primer was located upstream of the highly conserved P450 domain, this design distinguished whether the PCR products were P450 gene fragments depending on whether they contained the highly conserved amino acid domain.

[0086] All the fragments obtained from the PCR reaction were then cloned into a pUC19 vector, available from Stratagene, of LaJolla, Calif., and transformed into a DH5.alpha. E. coli strain, available from Gibco BRL, of Gaithersburg, Md.

[0087] Twenty-four positive colonies were obtained and sequenced. Sequence analysis indicated four groupings ~~withing~~ within the twenty-four colonies. One was ~~C411~~C4H, one was an unknown P450 gene, and two did not belong to P450 genes. Homologies of P450 genes in different species are usually more than 80%. Because the homologies between the P450 gene families found here were around 40%, the sequence analysis indicated that a new P450 gene family was sequenced. Moreover, since this P450 cDNA was isolated from xylem tissue, it was highly probable that this P450 gene was FA5HP450-1.

[0088] The novel sweetgum P450 cDNA fragment was used as a probe to screen a full length cDNA encoding for FA5HP450-1. Once the FA5HP450-1 gene was located it was sequenced. The length of the FA5HP450-1 cDNA is 1707 bp and it contains 45 bp of 5' non-coding region and 135 bp of 3' non-coding region. The deduced amino acid sequence also indicates that this P450 cDNA has a hydrophobic core at the N-terminal, which could be regarded as a leader sequence for c-translational targeting to membranes during protein synthesis. At the C-terminal region, there is a heme binding domain that is characteristic of all P450 genes. The FA5HP450-1 sequence, as illustrated in FIG. 4 (SEQ ID 1 and 2), was produced, according to the above described methods.

Example 4 - Isolating and Producing the DNA which Codes for the Angiosperm FA5HP450-2 Gene

[0089] By using similar strategy of synthesizing PCR primers from the published literature for hydroxylase genes in plants, another full length FA5HP450 cDNA has been isolated that shows significant similarity with a putative F5H clone from Arabidopsis (Meyers et al. 1996: PNAS 93, 6869-6874). This cloned cDNA, designated FA5HP450-2, contains 1883 bp and encodes an open reading frame of 511 amino acids. The amino acid similarity shared between Arabidopsis F5H FSH and the FA5HP450-2 sweetgum clone is about 75%, ~~indicating that the isolated clone belongs to the same class of cDNAs that encode a F5H protein, which has been shown to be functional by genetic complementation in Arabidopsis.~~

[0090] To confirm the function of the FA5HP450-2 gene, it was expressed in E.coli, strain, DH5 alpha, via pQE vector preparation, according to directions available with the kit. A CO--Fe2+ binding assay was also performed to confirm the expression of FA5HP450-2 as a functional P450 gene. (Omura & Sato 1964, J. of Biochemistry 239: 2370-2378, Babriac et.al. 1991 Archives of Biochemistry and Biophysics 288:302-309). The CO--Fe2+ binding assay showed a peak at 450 nm which indicates that FA5HP450-2 has been overexpressed as a functional P450 gene.

[0091] The FA5HP450-2 protein was further purified for production of antibodies in rabbits, and antibodies have been successfully produced. In addition, Western blots show that this antibody is specific to the membrane fraction of sweetgum and aspen xylem extract. When the FA5HP450-2 antibody was added to a reaction mixture containing aspen xylem tissue, enzyme inhibition studies showed that the activity of FA5HP450 in aspen was reduced more than 60%, a further indication that FA5H-2 performs a P450-like function. FIG. 5 (SEQ ID-2) illustrates the FA5H-2 sequence. P450-2 performs a p450like function. Recombinant P450-2 protein co-expressed with Arabidopsis CPR protein in a baculovirus expression system hydroxylated ferulic acid (specific activity: 7.3 pKat/mg protein), cinnaminic acid (specific activity: 25 pKat/mg protein, and p-coumeric acid (specific activity 3.8 pKat/ng protein). The P450-2 enzyme which may be referred to as C4C3F5-H appears to be a broad spectrum hydroxylase in the phenyproponoid pathway in plants FIG.5 (SEQ ID 3 and 4) illustrates the P450-2 sequence.

EXAMPLE 5 - Identifying Gymnosperm Promoter Regions

[0092] In order to identify gymnosperm promoter regions, sequences from loblolly pine PAL and 4CL1B-CL1B and 4CL3B lignin genes were used as primers to screen the loblolly pine genomic library, using the GenomeWalker Kit. The loblolly pine PAL primer sequence was obtained from the GenBank, reference number U39792. The loblolly pine 4CL1B primer sequences were also obtained from the gene bank, reference numbers U39404 and U39405.

[0093] The loblolly pine genomic library was constructed in Lambda DashII, available from Stratagene, of LaJolla, Calif. 3×10^6 phage plaques from the genomic library of loblolly pine were screened using both the above mentioned PAL cDNA and 4CL (PCR clone) fragments as probes. Five 4CL clones were obtained after screening. Lambda DNAs of two 4CL of the five 4CL clones obtained after screening were isolated and digested by EcoRV, PstHPstd, SalI-Sall and XbaI for Southern analysis. Southern analysis using 4CL fragments as

probes indicated that both clones for the 4CL gene were identical. Results from further mapping showed that none of the original five 4CL clones contained promoter regions. When tested, the PAL clones obtained from the screening also did not contain promoter regions.

[0094] In a second attempt to clone the promoter regions associated with the PAL and 4CL a Universal GenomeWalker.TM. kit, available from CLONETECH, was used. In the process, total DNA from loblolly pine was digested by several restriction enzymes and ligated into the adaptors (libraries) ~~previed~~ provided with the kit. Two gene-specific primers for each gene were designed (GSP1 and 2). After two rounds of PCR using these primers and adapter primers of the kit, several fragments were amplified from each library. A 1.6 kb fragment and a 0.6 kb fragment for PAL gene and a 2.3 kb fragment (4CL1B) and a 0.7 kb fragment (4CL3B) for the 4CL gene were cloned, sequenced and found to contain promoter regions for all three genes. See FIG. 6 (SEQ ID ~~610~~), 7 (SEQ ID ~~711~~) and 8 (SEQ ID ~~59~~).

Example 6 - Fusing the ASL DNA Sequence to A Constitutive Promoter Region and Inserting the Expression Cassette Into a Gymnosperm Genome

[0095] As a first step, a ASL DNA sequence, FA5HP450-1, was fused with a constitutive promoter region according to the methods described in the above Section IV to form an FA5HP450-1 expression cassette. A second ASL DNA sequence, FA5HP450-2, was then fused with a constitutive promoter in the same manner to form an FA5HP450-2 expression cassette. The FA5HP450-1 expression cassette was inserted into the gymnosperm genome by micro-projectile bombardment. Embryogenic tissue cultures of loblolly pine were initiated from immature zygotic embryos. The tissue was maintained in an undifferentiated state on semi-solid proliferation medium, according to methods described by Newton et al. TAES Technical Publication "Somatic Embryogenesis in Slash Pine", 1995 and Keinonen-Mettala et al. 1996, Scand. J. For. Res. 11: 242-250.

[0096] After separation, 5 ml of the liquid cell suspension fraction which passes through the 40 mesh screen was vacuum deposited onto filter paper and placed on semi-solid

proliferation medium. The prepared gymnosperm target cells were then grown for 2 days on filter paper discs placed on semi-solid proliferation medium in a petri dish. These target cell were then bombarded with plasmid DNA containing the FA5HP450-1 expression cassette and an expression cassette containing a selectable marker gene encoding the enzyme which confers resistance to the antibiotic hygromycin B. A 1:1 mixture of of selectable marker expression cassette and plasmid DNA containing the FA5HP450-1 expression cassette is precipitated with gold (1.5-3.0 microns) as described by Sanford et al. (1992). The DNA-coated microprojectiles were rinsed in absolute ethanol and ~~aliquots~~ aliquots of 10 µl (5 µg DNA/3 mg gold) were dried onto a macrocarrier, such as those available from BioRad (Hercules, Calif.).

[0097] Prior to bombardment, embryogenic tissue was desiccated under a sterile laminar-flow hood for 5 minutes. The desiccated tissue was transferred to semi-solid proliferation medium. The microprojectiles were accelerated into desiccated target cells using a BioRad PDS-1000/HE particle gun.

[0098] Each plate was bombarded once, rotated 180 degrees, and bombarded a second time. Preferred bombardment parameters were 1350 psi rupture disc pressure, 6 mm distance from the rupture disc to macrocarrier (gap distance), 1 cm macrocarrier travel distance, and 10 cm distance from macrocarrier stopping screen to culture plate (macrocarrier travel distance). Tissue was then transferred to semi-solid proliferation medium containing hygromycin B for two days after bombardment.

[0099] The FA5HP450-2 expression cassette was inserted into the gymnosperm genome according to the same procedures.

Example 7 - Selecting Transformed Target Cells

[0100] After insertion of the FA5HP450-1 expression cassette and the selectable marker expression cassette into the gymnosperm target cells as described in Example 6, transformed

cells were selected by exposure to an antibiotic that causes mortality of any cells not containing the GSL expression cassette. Forty independent cell lines were established from cultures cobombarded with an expression cassette containing a hygromycin resistance gene construct and the FA5HP450-1 construct. These cell lines include lines Y2, Y17, Y7 and 04, as discussed in more detail below.

[0101] PCR techniques were then used to verify that the FA5HP450-1 gene had been successfully integrated into the genomes of the established cell lines by extracting genomic DNA using the Plant DNAeasy kit, available from ~~Quaigen~~ Quaigen. 200 ng DNA from each cell line were used for each PCR reaction. Two FA5HP450-1 specific primers were designed to perform a PCR reaction with a 600 bp PCR product size. The primers were:

LsFA5HP450-im1-S primer: ATGGCTTTCCTTCTAATACCCATCTC (SEQ ID 23), and

LsFA5HP450-im1-A primer: GGGTGTAATGGACGAGCAAGGACTTG (SEQ ID 24).

[0102] Each PCR reaction (100 µl) consisted of 75 µl H₂O, 1 µl MgCl (25 mM), 10 µl PCR buffer 1 µl 10 mM dNTPs, and 10 µl DNA. 100 µl oil was layered on the top of each reaction mix. Hot start PCR was done as follows: PCR reaction was incubated at 95 degrees C. for 7 minutes and 1 µl each of both LsFA5HP450-im1-S and LsFA5HP450-im1-A primers (100 µM stock) and 1 µl of Taq polymerase were added through oil in each reaction. The PCR program used was 95 degrees C. for 1.5 minutes, 55 degrees C. for 45 sec and 72 degrees C. for 2 minutes, repeated for 40 cycles, followed by extension at 72 degrees C. for 10 minutes.

[0103] The above PCR products were employed to determine if gymnosperm cells contained the angiosperm lignin gene sequences. With reference to FIG. 9, PCR amplification was performed using template DNA from cells which grew vigorously on hygromycin B-containing medium. The PCR products were ~~electrophoresis~~ electrophoresed

in an agarose gel containing 9 lanes. Lanes 1-4 contained PCR amplification of products of the Sweetgum FA5HP450-1 gene from a non-transformed control and transgenic loblolly pine cell lines. Lane 1 contained the non-transformed control PT52. Lane 2 contained transgenic line Y2. Lane 3 contained transgenic line Y17 and Lane 4 contained the plasmid which contains the expression cassette pSSLs~~FA5HP4501~~-im-s. Lanes 2 through 4 all contain an amplified fragment of about 600 bp, indicating that the FA5HP450-1 gene has been successfully inserted into transgenic cell lines Y2 and Y17.

[0104] Lane 5 contained a DNA size marker Phi 174/~~HaeIII~~-HaeII (BRL). The top four bands in this lane indicate molecular sizes of 1353, 1078, 872 and 603 bp.

[0105] Lanes 6-9 contained PCR amplification products of hygromycin B gene from non-transformed control and transgenic loblolly pine cell lines. Lane 6 contained the non-transformed control ~~PT52 line, available from _____~~ lane referenced to as PTS. Lane 7 contained transgenic line Y7. Lane 8 contained transgenic line O4. Lane 9 contained the plasmid which includes the expression cassette containing the gene encoding the enzyme which confers resistance to the antibiotic hygromycin B. Lanes 7-9 all show an amplified fragment of about 1000 bp, indicating that the hygromycin gene has been successfully inserted into transgenic lines Y7 and O4.

[0106] These PCR results confirmed the presence of FA5HP450-1 and hygromycin resistance gene in transformed loblolly pine cell cultures. The results obtained from the PCR verification of 4 cell lines, and similar tests with the remaining 36 cell lines, confirm stable integration of the FA5HP450-1 gene and the hygromycin B gene in 25% of the 40 cell lines.

[0107] In addition, loblolly pine embryogenic cells which have been co-bombarded with the FA5HP450-2 and hygromycin B expression cassettes, are growing vigorously on hygromycin selection medium, indicating that the FA5HP450-2 expression cassette was successfully integrated into the gymnosperm genome.

[0108] Although various embodiments and features of the invention have been described in the foregoing detailed description, those of ordinary skill will recognize the invention is capable of numerous modifications, rearrangements and substitutions without departing from the scope of the invention as set forth in the appended claims. For example, in the case where the lignin DNA sequence is transcribed and translated to produce a functional syringyl lignin gene, those of ordinary skill will recognize that because of codon degeneracy a number of polynucleotide sequences will encode the same gene. These variants are intended to be covered by the DNA sequences disclosed and claimed herein. In addition, the sequences claimed herein include those sequences which encode a gene having substantial functional identity with those claimed. Thus, in the case of syringyl lignin genes, for example, the DNA sequences include variant polynucleotide sequences encoding polypeptides which have substantial identity with the amino acid sequence of syringyl lignin and which show syringyl lignin activity in gymnosperms.

"Sequence Listing"

Applicant: Chiang, et al
Title of Invention: PRODUCTION OF SYRINGYL LIGNIN
IN GYMNOSPERMS
Number of Sequences: 7
Information for Sequence ID #: 1 (FASH-1)
Correspondence Address: Luedeka, Neely & Graham
P.O. Box 1871
Knoxville, TN 37901
Addressee: Mark S. Graham
Street: P.O. Box 1871
City: Knoxville
Country: USA
Zip: 37901
Computer Readable Form:
Medium Type: 1.44
Operating System: DOS
Software: ASCII
Current Application Data: N/A
Attorney Information:
Name: Mark S. Graham
Registration Number: 32,355
Reference/Docket Number: 50617.00
Telecommunication Information:
Telephone: (423) 546-4305
Telefax: (423) 523-4478
Information for Seq ID No:
Sequence Characteristics:
Length: 1707
Type: DNA
Strandedness: double
Topology: linear
Molecule Type: cDNA
Hypothetical: No
Anti-Sense: No
Fragment Type: N/A
Original Source:
Organism: *Liquidambar styraciflua* (L.)
Strain: Wild Type
Individual Isolate: N/A
Developmental State: sporophyte
Haplotype: N/A
Tissue Type: xylem
Cell Type: parenchyma
Cell Line: N/A
Organelle: N/A

CGGCACGAGG AAACCCTAAA ACTCACCTCT CTTACCCTTT CTCTTCA ATG GCT TTC	56
Met Ala Phe	
CTT CTA ATA CCC ATC TCA ATA ATC TTC ATC GTC TTA GCT TAC CAG	101
Leu Leu Ile Phe Ile Ser Ile Ile Phe Ile Val Leu Ala Tyr Gln	
CTC TAT CAA CGG CTC AGA TTT AAG CTC CCA CCC GGC CCA CGT CCA	146
Leu Tyr Gln Arg Leu Arg Phe Lys Leu Pro Pro Gly Pro Arg Pro	
TGG CCG ATC GTC GGA AAC CTT TAC GAC ATA AAA CCG GTG AGG TTC	191
Trp Pro Ile Val Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg Phe	
CGG TGT TTC GCC GAG TGG TCA CAA GCG TAC GGT CCG ATC ATA TCG	236
Arg Cys Phe Ala Glu Trp Ser Gln Ala Tyr Gly Pro Ile Ile Ser	
GTG TGG TTC GGT TCA ACG TTG AAT GTG ATC GTA TCG AAT TCG GAA	281
Val Trp Phe Gly Ser Thr Leu Asn Val Ile Val Ser Asn Ser Glu	
TTG GCT AAG GAA GTG CTC AAG GAA AAA GAT CAA CAA TTG GCT GAT	326
Leu Ala Lys Glu Val Leu Lys Glu Lys Asp Gln Gln Leu Ala Asp	
AGG CAT AGG AGT AGA TCA GCT GCC AAA TTT AGC AGG GAT GGG CAG	371
Arg His Arg Ser Arg Ser Ala Ala Lys Phe Ser Arg Asp Gly Gln	
GAC CTT ATA TGG GCT GAT TAT GGA CCT CAC TAT GTG AAG GTT ACA	416
Asp Leu Ile Trp Ala Asp Tyr Gly Pro His Tyr Val Lys Val Thr	
AAG GTT TGT ACC CTC GAG CTT TTT ACT CCA AAG CGG CTT GAA GCT	461
Lys Val Cys Thr Leu Glu Leu Phe Thr Pro Lys Arg Leu Glu Ala	
CTT AGA CCC ATT AGA GAA GAT GAA GTT ACA GCC ATG GTT GAG TCC	506
Leu Arg Pro Ile Arg Glu Asp Glu Val Thr Ala Met Val Glu Ser	
ATT TTT AAT GAC ACT GCG AAT CCT GAA AAT TAT GGG AAG AGT ATG	551
Ile Phe Asn Asp Thr Ala Asn Pro Glu Asn Tyr Gly Lys Ser Met	
CTG GTG AAG AAG TAT TTG GGA GCA GTA GCA TTC AAC AAC ATT ACA	596
Leu Val Lys Lys Tyr Leu Gly Ala Val Ala Phe Asn Asn Ile Thr	
AGA CTC GCA TTT GGA AAG CGA TTC GTG AAT TCA GAG GGT GTA ATG	641
Arg Leu Ala Phe Gly Lys Arg Phe Val Asn Ser Glu Gly Val Met	
GAC GAG CAA GGA CTT GAA TTT AAG GAA ATT GTG GCC AAT GGA CTC	686
Asp Glu Gln Gly Leu Glu Phe Lys Glu Ile Val Ala Asn Gly Leu	
AAG CTT GGT GCC TCA CTT GCA ATG GCT GAG CAC ATT CCT TGG CTC	731
Lys Leu Gly Ala Ser Leu Ala Met Ala Glu His Ile Pro Trp Leu	
CGT TGG ATG TTC CCA CTT GAG GAA GGG GCC TTT GCC AAG CAT GGG	776
Arg Trp Met Phe Pro Leu Glu Glu Gly Ala Phe Ala Lys His Gly	
GCA CGT AGG GAC CGA CTT ACC AGA GCT ATC ATG GAA GAG CAC ACA	821
Ala Arg Arg Asp Arg Leu Thr Arg Ala Ile Met Glu Glu His Thr	

ATA GCC CGT AAA AAG AGT GGT GGA GCC CAA CAA CAT TTC GTG GAT Ile Ala Arg Lys Lys Ser Gly Gly Ala Gln Gln His Phe Val Asp	866
GCA TTG CTC ACC CTA CAA GAG AAA TAT GAC CTT AGC GAG GAC ACT Ala Leu Leu Thr Leu Gln Glu Lys Tyr Asp Leu Ser Glu Asp Thr	911
ATT ATT GGG CTC CTT TGG GAT ATG ATC ACT GCA GGC ATG GAC ACA Ile Ile Gly Leu Leu Trp Asp Met Ile Thr Ala Gly Met Asp Thr	956
ACC GCA ATC TCT GTC GAA TGG GCC ATG GCC GAG TTA ATT AAG AAC Thr Ala Ile Ser Val Glu Trp Ala Met Ala Glu Leu Ile Lys Asn	1001
CCA AGG GTG CAA CAA AAA GCT CAA GAG GAG CTA GAC AAT GTA CTT Pro Arg Val Gln Gln Lys Ala Gln Glu Glu Leu Asp Asn Val Leu	1046
GGG TCC GAA CGT GTC CTG ACC GAA TTG GAC TTC TCA AGC CTC CCT Gly Ser Glu Arg Val Leu Thr Glu Leu Asp Phe Ser Ser Leu Pro	1091
TAT CTA CAA TGT GTA GCC AAG GAG GCA CTA AGG CTG CAC CCT CCA Tyr Leu Gln Cys Val Ala Lys Glu Ala Leu Arg Leu His Pro Pro	1136
ACA CCA CTA ATG CTC CCT CAT CGC GCC AAT GCC AAC GTC AAA ATT Thr Pro Leu Met Leu Pro His Arg Ala Asn Ala Asn Val Lys Ile	1181
GGT GGC TAC GAC ATC CCT AAG GGA TCA AAT GTT CAT GTA AAT GTC Gly Gly Tyr Asp Ile Pro Lys Gly Ser Asn Val His Val Asn Val	1226
TGG GCC GTG GCT CGT GAT CCA GCA GTG TGG CGT GAC CCA CTA GAG Trp Ala Val Ala Arg Asp Pro Ala Val Trp Arg Asp Pro Leu Glu	1271
TTT CGA CCG GAA CGG TTC TCT GAA GAC GAT GTC GAC ATG AAA GGT Phe Arg Pro Glu Arg Phe Leu Glu Asp Asp Val Asp Met Lys Gly	1316
CAC GAT TAT AGG CTA CTG CCG TTT GGT GCA GGG AGG CGT GTT TGC His Asp Tyr Arg Leu Leu Pro Phe Gly Ala Gly Arg Arg Val Cys	1361
CCC GGT GCA CAA CTT GGC ATC AAT TTG GTC ACA TCC ATG ATG GGT Pro Gly Ala Gln Leu Gly Ile Asn Leu Val Thr Ser Met Met Gly	1406
CAC CTA TTG CAC CAT TTC TAT TGG AGC CCT CCT AAA GGT GTA AAA His Leu Leu His His Phe Tyr Trp Ser Pro Pro Lys Gly Val Lys	1451
CCA GAG GAG ATT GAC ATG TCA GAG AAT CCA GGA TTG GTC ACC TAC Pro Glu Glu Ile Asp Met Ser Glu Asn Pro Gly Leu Val Thr Tyr	1496
ATG CGA ACC CCG GTG CAA GCT GTT CCC ACT CCA AGG CTG CCT GCT Met Arg Thr Pro Val Gln Ala Val Pro Thr Pro Arg Leu Pro Ala	1541
CAC TTG TAC AAA CGT GTA GCT GTG GAT ATG TAATTCTTAG TTTGTTATTA His Leu Tyr Lys Arg Val Ala Val Asp Met	1591
TTCATGCTCT TAAGGTTTTG GACTTTGAAC TTATGATGAG ATTGTAAAA TTCCAAGTGA	1651
TCAAATGAAG AAAAGACCAA ATAAAAAGGC TTGACGATTT AAAAAAAAAA AAAAAA	1708

Information for Sequence ID #:	2 (FA5H-2)
Correspondence Address:	Luedeka, Neely & Graham P.O. Box 1871 Knoxville, TN 37901
Addressee:	Mark S. Graham
Street:	P.O. Box 1871
City:	Knoxville
Country:	USA
Zip:	37901
Computer Readable Form:	
Medium Type:	1.44
Operating System:	DOS
Software:	ASCII
Current Application Data:	N/A
Attorney Information:	
Name:	Mark S. Graham
Registration Number:	32,355
Reference/Docket Number:	50617.00
Telecommunication Information:	
Telephone:	(423) 546-4305
Telefax:	(423) 523-4478
Information for Seq ID No:	
Sequence Characteristics:	
Length:	1883
Type:	DNA
Strandedness:	double
Topology:	linear
Molecule Type:	cDNA
Hypothetical:	No
Anti-Sense:	No
Fragment Type:	N/A
Original Source:	
Organism:	<i>Liquidambar styraciflua</i> (L.)
Strain:	Wild Type
Individual Isolate:	N/A
Developmental State:	sporophyte
Haplotype:	N/A
Tissue Type:	xylem
Cell Type:	parenchyma
Cell Line:	N/A
Organelle:	N/A

TGCAAACCTG CACAAACAAA GAGAGAGAAG AAGAAAAAGG	40
AAGAGAGGAG AGAGAGAGAG AGAGAGAGAA GC	72
CAT GGA TTC TTC TCT TCA TGA AGC CTT GCA ACC ACT ACC CAT GAC GCT Met Asp Ser Ser Leu His Glu Ala Leu Gln Pro Leu Pro Met Thr Leu	120
GTT CTT CAT TAT ACC TTT GCT ACT CTT ATT GGG CCT AGT ATC TCG GCT Phe Phe Ile Ile Pro Leu Leu Leu Leu Leu Gly Leu Val Ser Arg Leu	168
TCG CCA GAG ACT ACC ATA CCC ACC AGG CCC AAA AGG CTT ACC GGT GAT Arg Gln Arg Leu Pro Tyr Pro Pro Gly Pro Lys Gly Leu Pro Val Ile	216
CGG AAA CAT GCT CAT GAT GGA TCA ACT CAC TCA CCG AGG ACT CGC CAA Gly Asn Met Leu Met Met Asp Gln Leu Thr His Arg Gly Leu Ala Lys	264
ACT CGC CAA ACA ATA CGG CGG TCT ATT CCA CCT CAA GAT GGG ATT CTT Leu Ala Lys Gln Tyr Gly Gly Leu Phe His Leu Lys Met Gly Phe Leu	312
ACA CAT GGT GGC CGT TTC CAC ACC CGA CAT GGC TCG CCA AGT CCT TCA His Met Val Ala Val Ser Thr Pro Asp Met Ala Arg Gln Val Leu Gln	360
AGT CCA AGA CAA CAT CTT CTC GAA CCG GCC AGC CAC CAT AGC CAT CAG Val Gln Asp Asn Ile Phe Ser Asn Arg Pro Ala Thr Ile Ala Ile Ser	408
CTA CCT CAC CTA TGA CCG AGC CGA CAT GGC CTT GGC TCA CTA CGG CCC Tyr Leu Thr Tyr Asp Arg Ala Asp Met Ala Phe Ala His Tyr Gly Pro	456
GTT TTG GCG TCA GAT GCG TAA ACT CTG CGT CAT GAA ATT ATT TAG CCG Phe Trp Arg Gln Met Arg Lys Leu Cys Val Met Lys Leu Phe Ser Arg	504
GAA ACG AGC CGA GTC GTG GGA GTC GGT CCG AGA CGA GGT CGA CTC GGC Lys Arg Ala Glu Ser Trp Glu Ser Val Arg Asp Glu Val Asp Ser Ala	552
AGT ACG AGT GGT CGC GTC CAA TAT TGG GTC GAC GGT GAA TAT CGG CGA Val Arg Val Val Ala Ser Asn Ile Gly Ser Thr Val Asn Ile Gly Glu	600
GCT GGT TTT TGC TCT GAC GAA GAA TAT TAC TTA CAG GGC GGC TTT TGG Leu Val Phe Ala Leu Thr Lys Asn Ile Thr Tyr Arg Ala Ala Phe Gly	648
GAC GAT CTC GCA TGA GGA CCA GGA CGA GTT CGT GGC CAT ACT GCA AGA Thr Ile Ser His Glu Asp Gln Asp Glu Phe Val Ala Ile Leu Gln Glu	696
GTT TTC GCA GCT GTT TGG TGC TTT TAA TAT AGC TGA TTT TAT CCC TTG	744

Phe Ser Gln Leu Phe Gly Ala Phe Asn Ile Ala Asp Phe Ile Pro Trp	
GCT CAA ATG GGT TCC TCA GGG GAT TAA CGT CAG GCT CAA CAA GGC ACG	792
Leu Lys Trp Val Pro Gln Gly Ile Asn Val Arg Leu Asn Lys Ala Arg	
AGG GGC GCT TGA TGG GTT TAT TGA CAA GAT CAT CGA CGA TCA TAT ACA	840
Gly Ala Leu Asp Gly Phe Ile Asp Lys Ile Ile Asp Asp His Ile Gln	
GAA GGG GAG TAA AAA CTC GGA GGA GGT TGA TAC TGA TAT GGT AGA TGA	888
Lys Gly Ser Lys Asn Ser Glu Glu Val Asp Thr Asp Met Val Asp Asp	
TTT ACT TGC TTT TTA CGG TGA GGA AGC CAA AGT AAG CGA ATC TGA CGA	936
Leu Leu Ala Phe Tyr Gly Glu Glu Ala Lys Val Ser Glu Ser Asp Asp	
TCT TCA AAA TTC CAT CAA ACT CAC CAA AGA CAA CAT CAA AGC TAT CAT	984
Leu Gln Asn Ser Ile Lys Leu Thr Lys Asp Asn Ile Lys Ala Ile Met	
GGA CGT AAT GTT TGG AGG GAC CGA AAC GGT GGC GTC CGC GAT TGA ATG	1032
Asp Val Met Phe Gly Gly Thr Glu Thr Val Ala Ser Ala Ile Glu Trp	
GGC CAT GAC GGA GCT GAT GAA AAG CCC AGA AGA TCT AAA GAA GGT CCA	1080
Ala Met Thr Glu Leu Met Lys Ser Pro Glu Asp Leu Lys Lys Val Gln	
ACA AGA ACT CGC CGT GGT GGT GGG TCT TGA CCC GCG AGT CGA AGA GAA	1128
Gln Glu Leu Ala Val Val Val Gly Leu Asp Arg Arg Val Glu Glu Lys	
AGA CTT CGA GAA GCT CAC CTA CTT GAA ATG CGT ACT GAA GGA AGT CCT	1176
Asp Phe Glu Lys Leu Thr Tyr Leu Lys Cys Val Leu Lys Glu Val Leu	
TCG CCT CCA CCC ACC CAT CCC ACT CCT CCT CCA CGA GAC TGC CGA GGA	1224
Arg Leu His Pro Pro Ile Pro Leu Leu Leu His Glu Thr Ala Glu Asp	
CGC CGA GGT CGG CGG CTA CTA CAT TCC GGC GAA ATC GCG GGT GAT GAT	1272
Ala Glu Val Gly Gly Tyr Tyr Ile Pro Ala Lys Ser Arg Val Met Ile	
CAA CGC GTG CGC CAT CGG CCG GGA CAA GAA CTC GTG GGC CGA CCC AGA	1320
Asn Ala Cys Ala Ile Gly Arg Asp Lys Asn Ser Trp Ala Asp Pro Asp	
TAC GTT TAG GCC CTC CAG GTT TCT CAA AGA CGG TGT GCC CGA TTT CAA	1368
Thr Phe Arg Pro Ser Arg Phe Leu Lys Asp Gly Val Pro Asp Phe Lys	
AGG GAA CAA CTT CGA GTT CAT CCC ATT CGG GTC AGG TCG TCG GTC TTG	1416
Gly Asn Asn Phe Glu Phe Ile Pro Phe Gly Ser Gly Arg Arg Ser Cys	

CCC CGG TAT GCA ACT CGG ACT CTA CGC GCT AGA GAC GAC TGT GGC TCA	1464
Pro Gly Met Gln Leu Gly Leu Tyr Ala Leu Glu Thr Thr Val Ala His	
CCT CCT TCA CTG TTT CAC GTG GGA GTT GCC GGA CGG GAT GAA ACC GAG	1512
Leu Leu His Cys Phe Thr Trp Glu Leu Pro Asp Gly Met Lys Pro Ser	
TGA ACT CGA GAT GAA TGA TGT GTT TGG ACT CAC CGC GCC AAG AGC GAT	1560
Glu Leu Glu Met Asn Asp Val Phe Gly Leu Thr Ala Pro Arg Ala Ile	
TCG ACT CAC CGC CGT GCC GAG TCC ACG CCT TCT CTG TCC TCT CTA	1605
Arg Leu Thr Ala Val Pro Ser Pro Arg Leu Leu Cys Pro Leu Tyr	
TTGATCGAAT GATTGGGGGA GCTTTGTGGA GGGGCTTTTA TGGAGACTCT ATATATAGAT	1665
GGGAAGTGAA ACAACGACAG GTGAATGCTT GGATTTTGG TATATATTGG GGAGGGAGGG	1725
GAAAAAAAAA ATAATGAAAG GAAAGAAAAG AGAGAATTTG AATTCTCTT CCTCTGTGGA	1785
TAAAGCCTC GTTTTAATT GTTTTATGT GGAGATATTT GTGTTGTTT ATTTTATCT	1845
CTTTTTTGC AATACACTC AAAAATAAAA AAAAAAA	1883

Information for Sequence ID #:	3 (bi-OMT)
Correspondence Address:	Luedeka, Neely & Graham
	P.O. Box 1871
	Knoxville, TN 37901
Addressee:	Mark S. Graham
Street:	P.O. Box 1871
City:	Knoxville
Country:	USA
Zip:	37901
Computer Readable Form:	
Medium Type:	1.44
Operating System:	DOS
Software:	ASCII
Current Application Data:	N/A
Attorney Information:	
Name:	Mark S. Graham
Registration Number:	32,355
Reference/Docket Number:	50617.00
Telecommunication Information:	
Telephone:	(423) 546-4305
Telefax:	(423) 523-4478
Information for Seq ID No:	
Sequence Characteristics:	
Length:	1380
Type:	DNA
Strandedness:	double
Topology:	linear
Molecule Type:	cDNA
Hypothetical:	No
Anti-Sense:	No
Fragment Type:	N/A
Original Source:	
Organism:	<i>Liquidambar styraciflua</i> (L.)
Strain:	Wild Type
Individual Isolate:	N/A
Developmental State:	sporophyte
Haplotype:	N/A
Tissue Type:	xylem
Cell Type:	parenchyma
Cell Line:	N/A
Organelle:	N/A

CGGCACGAGC CCTACCTCCT TTCTTGAAAA AATTTCCCCA TTCGATCACA ATCCGGGCCT	60
CAAAAA ATG GGA TCA ACA AGC GAA ACG AAG ATG AGC CCG AGT GAA GCA Met Gly Ser Thr Ser Glu Thr Lys Met Ser Pro Ser Glu Ala	108
GCA GCA GCA GAA GAA GAA GCA TTC GTA TTC GCT ATG CAA TTA ACC AGT Ala Ala Ala Glu Glu Glu Ala Phe Val Phe Ala Met Gln Leu Thr Ser	156
GCT TCA GTT CTT CCC ATG GTC CTA AAA TCA GCC ATA GAG CTC GAC GTC Ala Ser Val Leu Pro Met Val Leu Lys Ser Ala Ile Glu Leu Asp Val	204
TTA GAA ATC ATG GCT AAA GCT GGT CCA GGT GCG CAC ATA TCC ACA TCT Leu Glu Ile Met Ala Lys Ala Gly Pro Gly Ala His Ile Ser Thr Ser	252
GAC ATA GCC TCT AAG CTG CCC ACA AAG AAT CCA GAT GCA GCC GTC ATG Asp Ile Ala Ser Lys Leu Pro Thr Lys Asn Pro Asp Ala Ala Val Met	300
CTT GAC CGT ATG CTC CGC CTC TTG GCT AGC TAC TCT GTT CTA ACG TGC Leu Asp Arg Met Leu Arg Leu Leu Ala Ser Tyr Ser Val Leu Thr Cys	348
TCT CTC CGC ACC CTC CCT GAC GGC AAG ATC GAG AGG CTT TAC GGC CTT Ser Leu Arg Thr Leu Pro Asp Gly Lys Ile Glu Arg Leu Tyr Gly Leu	396
GCA CCC GTT TGT AAA TTC TTG ACC AGA AAC GAT GAT GGA GTC TCC ATA Ala Pro Val Cys Lys Phe Leu Thr Arg Asn Asp Asp Gly Val Ser Ile	444
GCC GCT CTG TCT CTC ATG AAT CAA GAC AAG GTC CTC ATG GAG AGC TGG Ala Ala Leu Ser Leu Met Asn Gln Asp Lys Val Leu Met Glu Ser Trp	492
TAC CAC TTG ACC GAG GCA GTT CTT GAA GGT GGA ATT CCA TTT AAC AAG Tyr His Leu Thr Glu Ala Val Leu Glu Gly Gly Ile Pro Phe Asn Lys	540
GCC TAT GGA ATG ACA GCA TTT GAG TAC CAT GGC ACC GAT CCC AGA TTC Ala Tyr Gly Met Thr Ala Phe Glu Tyr His Gly Thr Asp Pro Arg Phe	588
AAC ACA GTT TTC AAC AAT GGA ATG TCC AAT CAT TCG ACC ATT ACC ATG Asn Thr Val Phe Asn Asn Gly Met Ser Asn His Ser Thr Ile Thr Met	636
AAG AAA ATC CTT GAG ACT TAC AAA GGG TTC GAG GGA CTT GGA TCT GTG Lys Lys Ile Leu Glu Thr Tyr Lys Gly Phe Glu Gly Leu Gly Ser Val	684
GTT GAT GTT GGT GGT GGC ACT GGT GCC CAC CTT AAC ATG ATT ATC GCT Val Asp Val Gly Gly Gly Thr Gly Ala His Leu Asn Met Ile Ile Ala	732
AAA TAC CCC ATG ATC AAG GGC ATT AAC TTC GAC TTG CCT CAT GTT ATT Lys Tyr Pro Met Ile Lys Gly Ile Asn Phe Asp Leu Pro His Val Ile	780
GAG GAG GCT CCC TCC TAT CCT GGT GTG GAG CAT GTT GGT GGA GAT ATG Glu Glu Ala Pro Ser Tyr Pro Gly Val Glu His Val Gly Gly Asp Met	828
TTT GTT AGT GTT CCA AAA GGA GAT GCC ATT TTC ATG AAG TGG ATA TGT Phe Val Ser Val Pro Lys Gly Asp Ala Ile Phe Met Lys Trp Ile Cys	876

CAT GAT TGG AGC GAT GAA CAC TGC TTG AAG TTT TTG AAG AAA TGT TAT	924
His Asp Trp Ser Asp Glu His Cys Leu Lys Phe Leu Lys Lys Cys Tyr	
GAA GCA CTT CCA ACC AAT GGG AAG GTG ATC CTT GCT GAA TGC ATC CTC	972
Glu Ala Leu Pro Thr Asn Gly Lys Val Ile Leu Ala Glu Cys Ile Leu	
CCC GTG GCG CCA GAC GCA AGC CTC CCC ACT AAG GCA GTG GTC CAT ATT	1020
Pro Val Ala Pro Asp Ala Ser Leu Pro Thr Lys Ala Val Val His Ile	
GAT GTC ATC ATG TTG GCT CAT AAC CCA GGT GGG AAA GAG AGA ACT GAG	1068
Asp Val Ile Met Leu Ala His Asn Pro Gly Gly Lys Glu Arg Thr Glu	
AAG GAG TTT GAG GCC TTG GCC AAG GGG GCT GGA TTT GAA GGT TTC CGA	1116
Lys Glu Phe Glu Ala Leu Ala Lys Gly Ala Gly Phe Glu Gly Phe Arg	
GTA GTA GCC TCG TGC GCT TAC AAT ACA TGG ATC ATC GAA TTT TTG AAG	1164
Val Val Ala Ser Cys Ala Tyr Asn Thr Trp Ile Ile Glu Phe Leu Lys	
AAG ATT TGAGTCCTTA CTCGGCTTTG AGTACATAAT ACCAACTCCT TTTGGTTTTT	1220
Lys Ile	
GAGATTGTGA TTGTGATTGT GATTGTCTCT CTTTCGCAGT TGGCCTTATG ATATAATGTA	1280
TCGTTAACTC GATCACAGAA GTGCAAAAGA CAGTGAATGT ACACTGCTTT ATAAAATAAA	1340
AATTTTAAGA TTTTGATTCA TGTAAAAAAA AAAAAAAAAA	1380

Information for Sequence ID #: 4 (4CL)
Correspondence Address: Luedeka, Neely & Graham
P.O. Box 1871
Knoxville, TN 37901
Addressee: Mark S. Graham
Street: P.O. Box 1871
City: Knoxville
Country: USA
Zip: 37901
Computer Readable Form:
Medium Type: 1.44
Operating System: DOS
Software: ASCII
Current Application Data: N/A
Attorney Information:
Name: Mark S. Graham
Registration Number: 32,355
Reference/Docket Number: 50617.00
Telecommunication Information:
Telephone: (423) 546-4305
Telefax: (423) 523-4478
Information for Seq ID No:
Sequence Characteristics:
Length: 2026
Type: DNA
Strandedness: double
Topology: linear
Molecule Type: cDNA
Hypothetical: No
Anti-Sense: No
Fragment Type: N/A
Original Source:
Organism: *Liquidambar styraciflua* (L.)
Strain: Wild Type
Individual Isolate: N/A
Developmental State: sporophyte
Haplotype: N/A
Tissue Type: xylem
Cell Type: parenchyma
Cell Line: N/A
Organelle: N/A

CGGCACGAGC TCATTTTCCA CTTCTGGTTT GATCTCTGCA ATTCTTCCAT CAGTCCCTA	60
ATG GAG ACC CAA ACA AAA CAA GAA GAA ATC ATA TAT CGG TCG AAA	105
Met Glu Thr Gln Thr Lys Gln Glu Glu Ile Ile Tyr Arg Ser Lys	
CTC CCC GAT ATC TAC ATC CCC AAA CAC CTC CCT TTA CAT TCG TAT	150
Leu Pro Asp Ile Tyr Ile Pro Lys His Leu Pro Leu His Ser Tyr	
TGT TTC GAG AAC ATC TCA CAG TTC GGC TCC CGC CCC TGT CTG ATC	195
Cys Phe Glu Asn Ile Ser Gln Phe Gly Ser Arg Pro Cys Leu Ile	
AAT GGC GCA ACG GGC AAG TAT TAC ACA TAT GCT GAG GTT GAG CTC	240
Asn Gly Ala Thr Gly Lys Tyr Tyr Thr Tyr Ala Glu Val Glu Leu	
ATT GCG CGC AAG GTC GCA TCC GGC CTC AAC AAA CTC GGC GTT CGA	285
Ile Ala Arg Lys Val Ala Ser Gly Leu Asn Lys Leu Gly Val Arg	
CAA GGT GAC ATC ATC ATG CTT TTG CTA CCC AAC TCG CCG GAG TTC	330
Gln Gly Asp Ile Ile Met Leu Leu Leu Pro Asn Ser Pro Glu Phe	
GTG TTT TCA ATT CTC GGC GCA TCC TAC GGC GGG GCT GCC GCC ACC	375
Val Phe Ser Ile Leu Gly Ala Ser Tyr Arg Gly Ala Ala Ala Thr	
GCC GCA AAC CCG TTT TAT ACC CCT GCC GAG ATC AGG AAG CAA GCC	420
Ala Ala Asn Pro Phe Tyr Thr Pro Ala Glu Ile Arg Lys Gln Ala	
AAA ACC TCC AAC GCC AGG CTT ATT ATC ACA CAT GCC TGT TAC TAT	465
Lys Thr Ser Asn Ala Arg Leu Ile Ile Thr His Ala Cys Tyr Tyr	
GAG AAA GTG AAG GAC TTG GTG GAA GAG AAC GTT GCC AAG ATC ATA	510
Glu Lys Val Lys Asp Leu Val Glu Glu Asn Val Ala Lys Ile Ile	
TGT ATA GAC TCA CCC CCG GAC GGT TGT TTG CAC TTC TCG GAG CTG	555
Cys Ile Asp Ser Pro Pro Asp Gly Cys Leu His Phe Ser Glu Leu	
AGT GAG GCG GAC GAG AAC GAC ATG CCC AAT GTA GAG ATT GAC CCC	600
Ser Glu Ala Asp Glu Asn Asp Met Pro Asn Val Glu Ile Asp Pro	
GAT GAT GTG GTG GCG CTG CCG TAC TCG TCA GGG ACG ACG GGT TTA	645
Asp Asp Val Val Ala Leu Pro Tyr Ser Ser Gly Thr Thr Gly Leu	
CCA AAG GGG GTG ATG CTA ACA CAC AAG GGA CAA GTG ACG AGT GTG	690
Pro Lys Gly Val Met Leu Thr His Lys Gly Gln Val Thr Ser Val	
GCG CAA CAG GTG GAC GGA GAG AAT CCG AAC CTG TAT ATA CAT AGC	735
Ala Gln Gln Val Asp Gly Glu Asn Pro Asn Leu Tyr Ile His Ser	
GAG GAC GTG GTT CTG TGC GTG TTG CCT CTG TTT CAC ATC TAC TCG	780
Glu Asp Val Val Leu Cys Val Leu Pro Leu Phe His Ile Tyr Ser	
ATG AAC GTC ATG TTT TGC GGG TTA CGA GTT GGT GCG GCG ATT CTG	825
Met Asn Val Met Phe Cys Gly Leu Arg Val Gly Ala Ala Ile Leu	
ATT ATG CAG AAA TTT GAA ATA TAT GGG TTG TTA GAG CTG GTC AGA	870
Ile Met Gln Lys Phe Glu Ile Tyr Gly Leu Leu Glu Leu Val Arg	
AGT ACA GGT GAC CAT CAT GCC TAT CGT ACA CCC ATC GTA TTG GCA	915

Ser Thr Gly Asp His His Ala Tyr Arg Thr Pro Ile Val Leu Ala	
ATC TCC AAG ACT CCG GAT CTT CAC AAC TAT GAT GTG TCC TC ATT	960
Ile Ser Lys Thr Pro Asp Leu His Asn Tyr Asp Val Ser Ser Ile	
CGG ACT GTC ATG TCA GGT GCG GCT CCT CTG GGC AAG GAA CT GAA	1005
Arg Thr Val Met Ser Gly Ala Ala Pro Leu Gly Lys Glu Leu Glu	
GAT TCT GTC AGA GCT AAG TTT CCC ACC GCC AAA CTT GGT CAG GGA	1050
Asp Ser Val Arg Ala Lys Phe Pro Thr Ala Lys Leu Gly Gln Gly	
TAT GGA ATG ACG GAG GCA GGG CCC GTG CTA GCG ATG TGT TTG CA	1095
Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala Met Cys Leu Ala	
TTT GCC AAG GAA GGG TTT GAA ATA AAA TCG GGG GCA TCT GGA AT	1140
Phe Ala Lys Glu Gly Phe Glu Ile Lys Ser Gly Ala Ser Gly Thr	
GTT TTA AGG AAC GCA CAG ATG AAG ATT GTG GAC CCT GAA ACC GG	1185
Val Leu Arg Asn Ala Gln Met Lys Ile Val Asp Pro Glu Thr Gly	
GTC ACT CTC CCT CGA AAC CAA CCC GGA GAG ATT TGC ATT AGA GGA	1230
Val Thr Leu Pro Arg Asn Gln Pro Gly Glu Ile Cys Ile Arg Gly	
GAC CAA ATC ATG AAA GGT TAT CTT AAT GAT CCT GAG GCG ACG GAG	1275
Asp Gln Ile Met Lys Gly Tyr Leu Asn Asp Pro Glu Ala Thr Glu	
AGA ACC ATA GAC AAG GAA GGT TGG TTA CAC ACA GGT GAT GTG GGC	1320
Arg Thr Ile Asp Lys Glu Gly Trp Leu His Thr Gly Asp Val Gly	
TAC ATC GAC GAT GAC ACT GAG CTC TTC ATT GTT GAT CGG TTG AAG	1365
Tyr Ile Asp Asp Asp Thr Glu Leu Phe Ile Val Asp Arg Leu Lys	
GAA CTG ATC AAA TAC AAA GGG TTT CAG GTG GCA CCC GCT GAG CTT	1410
Glu Leu Ile Lys Tyr Lys Gly Phe Gln Val Ala Pro Ala Glu Leu	
GAG GCC ATG CTC CTC AAC CAT CCC AAC ATC TCT GAT GCT GCC GTC	1455
Glu Ala Met Leu Leu Asn His Pro Asn Ile Ser Asp Ala Ala Val	
GTC CCA ATG AAA GAC GAT GAA GCT GGA GAG CTC CCT GTG GCG TTT	1500
Val Pro Met Lys Asp Asp Glu Ala Gly Glu Leu Pro Val Ala Phe	
GTT GTA AGA TCA GAT GGT TCT CAG ATA TCC GAG GCT GAA ATC AGG	1545
Val Val Arg Ser Asp Gly Ser Gln Ile Ser Glu Ala Glu Ile Arg	
CAA TAC ATC GCA AAA CAG GTG GTT TTT TAT AAA AGA ATA CAT CGC	1590
Gln Tyr Ile Ala Lys Gln Val Val Phe Tyr Lys Arg Ile His Arg	
GTA TTT TTC GTC GAA GCC ATT CCT AAA GCG CCC TCT GGC AAA ATC	1635
Val Phe Phe Val Glu Ala Ile Pro Lys Ala Pro Ser Gly Lys Ile	
TTG CGG AAG GAC CTG AGA GCC AAA TTG GCG TCT GGT CTT CCC AAT	1680
Leu Arg Lys Asp Leu Arg Ala Lys Leu Ala Ser Gly Leu Pro Asn	
TAATTCTCAT TCGCTACCCT CCTTCTCTT ATCATACGCC AACACGAACG AAGAGGCTCA	1740
ATTAAACGCT GCTCATTCGA AGCGGCTCAA TTAAAGCTGC TCATTCATGT CCACCGAGTG	1800

GGCAGCCTGT CTTGTTGGGA TGTTCTTTCA TTTGATTGAG CTGTGAGAAG CCAGACCCTC 1860
ATTATTTATT GTGAAATTCA CAAGAATGTC TGTAATCGA TGTTGTGAGT GATGGGTTTC 1920
AAAACACTTT TGACATTGTT TACGTTGTAT TTCCTGCTGT TGAAAATAAC TACTTTGTAT 1980
GACTTTTATT TGGGAAGATA ACCTTTCAAA AAAAAAAAAA AAAAAA 2026

Information for Sequence ID #:	5 (PAL)
Correspondence Address:	Luedeka, Neely & Graham
	P.O. Box 1871
	Knoxville, TN 37901
Addressee:	Mark S. Graham
Street:	P.O. Box 1871
City:	Knoxville
Country:	USA
Zip:	37901
Computer Readable Form:	
Medium Type:	1.44
Operating System:	DOS
Software:	ASCII
Current Application Data:	N/A
Attorney Information:	
Name:	Mark S. Graham
Registration Number:	32,355
Reference/Docket Number:	50617.00
Telecommunication Information:	
Telephone:	(423) 546-4305
Telefax:	(423) 523-4478
Information for Seq ID No:	
Sequence Characteristics:	
Length:	1544
Type:	DNA
Strandedness:	double
Topology:	linear
Molecule Type:	cDNA
Hypothetical:	No
Anti-Sense:	No
Fragment Type:	N/A
Original Source:	
Organism:	Pinus taeda (L.)
Strain:	Wild Type
Individual Isolate:	N/A
Developmental State:	sporophyte
Haplotype:	N/A
Tissue Type:	xylem
Cell Type:	parenchyma
Cell Line:	N/A
Organelle:	N/A

AAAGATAATA TATGTGTATG CCTACTACTA CACATTGTTT TGAAGTGTGT AAACATAGTG	60
CAACACTAGG AGGACTCACA ATGAGCACCT GTTGACATGA AACTAGCTAA ATGCCCAACA	120
ATATTAGTGA AAGCTAGTTA AACTAACCCC TTTGACTTTC AAGATGATAT ATTTATATCC	180
CTACTACGTC TTCTCTTTT TGTCTTCTC TTGTGATTAA ACCTTCCTTG AAACAATTCT	240
CAAATGTAAA ATTAAACCTT GAAACTTGTA GAGACCAAAC TTCCCTAGGA GAAACCACAT	300
TTATGACAAC ATATATACAC CAACCCATTG CATACTATAA TATTGGAATT ACCTGCAGCG	360
AACGAAAGAA ACGCTGTCTC ACCAACTCGT GCACTACATC CCGAAACTTA ACCTTCCCCT	420
GATACAGATT GAAGAGCCGA AAAAAGCGTG CATCCAAATT TCTGGTATGG TGAGGAGCCG	480
AAAAACGCGT GCGCCTAATT TTTTGTAGAT GGGCCGAAA ATAATGCGTG CATCTAAATT	540
TTCACGTGTC GCGTATTGGC GAGGTTGCGC TGAATGTGAT CCTGTGCGTG AGCCACATTC	600
ATTCCATTGG TTGACCCGCC GGTACCGCGA GGACCGTGGG GTCTCACAGA TACGCGGATG	660
GTGGATCAGC ACTGAGAAGA TTAGATGATG ACCAGGCGGG CATTTGAAGT AAAAATTGG	720
GGGTGGTTGG CAAGTACGCG ACAAAGAGGG GTAGTGCGCA AGGAAGCGAG TTGGATGCAA	780
ATAATATTAC AAAGTGGGTT GGTGGGCATG AGCATCAACC AGAATGATGT TGTGCTGGT	840
TCCGTGCAAA TTCTGACCAG TAGTTGAAC AATACTACCC AACTTGTTTT TGGTAAAACA	900
TGAAGTGGGT AAGGAGAATT GAACTTACGT CTCATGGTAA AGGCAAGGG CAAATGACTT	960
AACACATACC TTAACTAAT AAAAATACCC CTAACAAATA CGAAAACGAA TGAGTTATCA	1020
CAGACCTTCA ACTAATAAGA TAGCCATCAG ACCCACATCT CTGACTGAC CAAAAACAA	1080
TGACTTCAAC CAACTAAGAT ACCCATCAAA GCTAACCAC AACCCAATTC CTCACTTCCC	1140
CTTACCAGAC CAACCAAGCA GACCTACGCC ATTAATACT TTAGGACGTG GGAATTGGGG	1200
GTGCCACCGT TGAAGAATGG CACTCAGGGT TGGTAATCCC TCCACGTGTA TGTCAGATC	1260
TTTTGGTGGA GACGGCGTGT TTGAATGTCC ACCTTCCAGT TTGGAGAACA AGGAAATGG	1320
GCTTATATTA GGCCTGGATC TCTTGTTC A GAGCAGGAGT AGTTCAGGAC AGGAACTAGC	1380
ATTCAAGAAT TCAATTGCCC TGCCCTGCTC TGCTCTGCTT TGCTCAACTT ATTGATCCCT	1440
GCTCTGGTTT GTTCAATTC TTGACCCCTG CTGGGTTCTG CTCTGGTTTG CACACTTTCT	1500
CGATTATATA AGTCATTTTG GATCCTTGCA AGGAAGAGAA TATG	1544

Information for Sequence ID #:	6 (4CL1B)
Correspondence Address:	Luedeka, Neely & Graham
	P.O. Box 1871
	Knoxville, TN 37901
Addressee:	Mark S. Graham
Street:	P.O. Box 1871
City:	Knoxville
Country:	USA
Zip:	37901
Computer Readable Form:	
Medium Type:	1.44
Operating System:	DOS
Software:	ASCII
Current Application Data:	N/A
Attorney Information:	
Name:	Mark S. Graham
Registration Number:	32,355
Reference/Docket Number:	50617.00
Telecommunication Information:	
Telephone:	(423) 546-4305
Telefax:	(423) 523-4478
Information for Seq ID No:	
Sequence Characteristics:	
Length:	659
Type:	DNA
Strandedness:	double
Topology:	linear
Molecule Type:	cDNA
Hypothetical:	No
Anti-Sense:	No
Fragment Type:	N/A
Original Source:	
Organism:	Pinus taeda (L.)
Strain:	Wild Type
Individual Isolate:	N/A
Developmental State:	sporophyte
Haplotype:	N/A
Tissue Type:	xylem
Cell Type:	parenchyma
Cell Line:	N/A
Organelle:	N/A

AAACACCAAT TTAATGGGAT TTCAGATTTG TATCCCATGC TATTGGCTAA GGCATTTTTC	60
TTATNGTAAT CTAACCAATT CTAATTTCCA CCCTGGTGTG AACTGACTGA CAAATGCGGT	120
CCGAAAACAG CGAATGAAAT GTCTGGGTGA TCGGTCAAAC AAGCGGTGGG CGAGAGAGCG	180
CGGGTGTGG CCTAGCCGGG ATGGGGGTAG GTAGACGGCG TATTACCGGC GAGTTGTCCG	240
AATGGAGTTT TCGGGGTAGG TAGTAACGTA GACGTCAATG GAAAAAGTCA TAATCTCCGT	300
CAAAAATCCA ACCGCTCCTT CACATCGCAG AGTTGGTGCC CACGGGACCC TCCACCCACT	360
CACTCAATCG ATCGCCTGCC GTGGTTGCCC ATTATTCAAC CATACGCCAC TTGACTCTTC	420
ACCAACAATT CCAGGCCGGC TTTCTATACA ATGTACTGCA CAGGAAAATC CAATATAAAA	480
AGCCGGCCTC TGCTTCCTTC TCAGTAGCCC CCAGCTCATT CAATTCTTCC CACTGCAGGC	540
TACATTGTC AGACACGTTT TCCGCCATTT TTCGCCTGTT TCTGCGGAGA ATTGATCAG	600
GTTCCGATTG GGATTGAATC AATTGAAAGG TTTTATTTT CAGTATTTG ATCGCCATG	659

Information for Sequence ID #: 7 (4CL3B)
 Correspondence Address: Luedeka, Neely & Graham
 P.O. Box 1871
 Knoxville, TN 37901
 Addressee: Mark S. Graham
 Street: P.O. Box 1871
 City: Knoxville
 Country: USA
 Zip: 37901
 Computer Readable Form:
 Medium Type: 1.44
 Operating System: DOS
 Software: ASCII
 Current Application Data: N/A
 Attorney Information:
 Name: Mark S. Graham
 Registration Number: 32,355
 Reference/Docket Number: 50617.00
 Telecommunication Information:
 Telephone: (423) 546-4305
 Telefax: (423) 523-4478
 Information for Seq ID No:
 Sequence Characteristics:
 Length: 2251
 Type: DNA
 Strandedness: double
 Topology: linear
 Molecule Type: cDNA
 Hypothetical: No
 Anti-Sense: No
 Fragment Type: N/A
 Original Source:
 Organism: Pinus taeda (L.)
 Strain: Wild Type
 Individual Isolate: N/A
 Developmental State: sporophyte
 Haplotype: N/A
 Tissue Type: xylem
 Cell Type: parenchyma
 Cell Line: N/A
 Organelle: N/A

GGCCGGGTGG TGACATTTAT TCATAAATTC ATCTCAAAAC AAGAAGGATT TACAAAAATA	60
AAAGAAACA AAATTTTCAT CTTAACATA ATTATAATTG TGTTACAAA ATTCAAACCTT	120
AAACCCTTAA TATAAAGAAT TTCTTTCAAC AATACACTTT AATCACAACT TCTTCAATCA	180
CAACCTCCTC CAACAAAATT AAAATAGATT AATAAATAA TAAACTTAAC TATTTAAAAA	240
AAAATATTAT AAAAAATTTA TTAAACTTC AAAATAACA AACTTTTTAT ACAAATTCA	300
TCAAACTTT AAAATAAGC TAAACACTGA AAATGTGAGT ACATTTAAAA GGACGCTGAT	360
CACAAAATT TTGAAACAT AAACAACTT GAACTCTAC CTTTAAGAA TGAGTTTGTC	420
GTCTCATTAA CTCATTAGTT TTATAGTTCG AATCCAATTA ACGTATCTTT TATTTTATGG	480
AATAAGGGTG TTTAATAAG TGTTTTGGG ATTTTTTAG TAATTTATT GTGATATGTT	540
ATGGAGTTTT TAAAAATATA TATATATATA TATATTTTG GGTGAGTTT ACTTAAATT	600
TGGAAAAGGT TGGTAAGAAC TATAAATTGA GTTGTGAATG AGTGTTTTAT GGATTTTTTA	660
AGATGTTAAA TTTATATATG TAATTAAAT TTTATTTGA ATAACAAAA TTATAATTGG	720
ATAAAAAATT GTTTTGTAA ATTTAGAGTA AAATTTCAA AATCTAAAT AATTAAACAC	780
TATTATTTT AAAAAATTG TTGGTAAAT TTATCTATA TTTAAGTTAA AATTTAGAAA	840
AAATTAATTT TAAATTAATA AACTTTTGAA GTCAAATAT CCAATATTT TCCAAAATAT	900
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CAATTTGTAT AAAAACCAA AATCTCAAAT AAAATTAAA TTACAAAACA TTATCAACAT	1020
TATGATTTCA AGAAAGACAA TAACCAGTTT CCAATAAAAT AAAAAACCTC ATGGCCCGTA	1080
ATTAAGATCT CATTAAATTA TTCTTATTT TTAATTTTT TACATAGAAA ATATCTTTAT	1140
ATTGTATCCA AGAAATATAG AATGTTCTCG TCCAGGGACT ATTAATCTCC AAACAAGTTT	1200
CAAAATCATT ACATTAAAGC TCATCATGTC ATTTGTGGAT TGGAAATTAT ATTGTATAAG	1260
AGAAATATAG AATGTTCTCG TCTAGGGACT ATTAATTTCC AAACAAATTT CAAATCATT	1320
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TTCTCTCAAT CTCCAAAAT ATAGTTCGAA CTCCATATTT TTGGAAATTG AGAATTTTTT	1440
TACCCAATAA TATATTTTTT TATACATTTT AGAGATTTTC CAGACATATT TGCTCTGGGA	1500
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AGATACTAAA TCCATTATAT AATAAAAACA CATTTTAAAC ACCAATTAA TGGGATTTCA	1620
GATTTGTATC CCATGCTATT GGCTAAGGCA TTTTCTTAT TGTAATCTAA CCAATTCTAA	1680

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GGGTAGGTAG ACGSCGTATT ACCGGCGAGT TGTCCGAATG GAGTTTTCGG GGTAGGTAGT	1860
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TCGCAGAGTT GGTGGCCACG GGACCTCCA CCCACTCACT CGATCGCCTG CCGTGGTTGC	1980
CCATTATTCA ACCATACGCC ACTTGACTCT TCACCAACAA TTCCAGGCCG GCTTTCTATA	2040
CAATGTACTG CACAGGAAAA TCCAATATAA AAAGCCGGCC TCTGCTTCCT TCTCAGTAGC	2100
CCCCAGCTCA TTCAATTCTT CCCACTGCAG GCTACATTG TCAGACACGT TTTCCGCCAT	2160
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SEQUENCE LISTING

<110> CHIANG, VINCENT L.

CARRAWAY, DANIEL T.

SMELTZER, RICHARD H.

<120> PRODUCTION OF SYRINGYL LIGNIN IN GYMNOSPERMS

<130> 044463-0336

<140> 10/681,878

<141> 2003-10-09

<150> 09/796,256

<151> 2001-02-28

<150> 08/991,677

<151> 1997-12-16

<150> 60/033,381

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Leu Leu Ile Pro Ile Ser Ile Ile Phe Ile Val Leu Ala Tyr Gln Leu
5 10 15
tat caa cgg ctc aga ttt aag ctc cca ccc ggc cca cgt cca tgg ccg 152
Tyr Gln Arg Leu Arg Phe Lys Leu Pro Pro Gly Pro Arg Pro Trp Pro
20 25 30 35
atc gtc gga aac ctt tac gac ata aaa ccg gtg agg ttc cgg tgt ttc 200
Ile Val Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg Phe Arg Cys Phe
40 45 50
gcc gag tgg tca caa gcg tac ggt ccg atc ata tcg gtg tgg ttc ggt 248
Ala Glu Trp Ser Gln Ala Tyr Gly Pro Ile Ile Ser Val Trp Phe Gly
55 60 65
tca acg ttg aat gtg atc gta tcg aat tcg gaa ttg gct aag gaa gtg 296
Ser Thr Leu Asn Val Ile Val Ser Asn Ser Glu Leu Ala Lys Glu Val
70 75 80
ctc aag gaa aaa gat caa caa ttg gct gat agg cat agg agt aga tca 344
Leu Lys Glu Lys Asp Gln Gln Leu Ala Asp Arg His Arg Ser Arg Ser
85 90 95
gct gcc aaa ttt agc agg gat ggg cag gac ctt ata tgg gct gat tat 392
Ala Ala Lys Phe Ser Arg Asp Gly Gln Asp Leu Ile Trp Ala Asp Tyr
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gga cct cac tat gtg aag gtt aca aag gtt tgt acc ctc gag ctt ttt 440
Gly Pro His Tyr Val Lys Val Thr Lys Val Cys Thr Leu Glu Leu Phe

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<u>ggt gta atg gac gag caa gga ctt gaa ttt aag gaa att gtg gcc aat 680</u>			
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<u>Gly Leu Lys Leu Gly Ala Ser Leu Ala Met Ala Glu His Ile Pro Trp</u>			
215	220	225	
<u>ctc cgt tgg atg ttc cca ctt gag gaa ggg gcc ttt gcc aag cat ggg 776</u>			
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230	235	240	
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245	250	255	

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Ala Arg Lys Lys Ser Gly Gly Ala Gln Gln His Phe Val Asp Ala Leu

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ctc acc cta caa gag aaa tat gac ctt agc gag gac act att att ggg 920

Leu Thr Leu Gln Glu Lys Tyr Asp Leu Ser Glu Asp Thr Ile Ile Gly

280 285 290

ctc ctt tgg gat atg atc act gca ggc atg gac aca acc gca atc tct 968

Leu Leu Trp Asp Met Ile Thr Ala Gly Met Asp Thr Thr Ala Ile Ser

295 300 305

gtc gaa tgg gcc atg gcc gag tta att aag aac cca agg gtg caa caa 1016

Val Glu Trp Ala Met Ala Glu Leu Ile Lys Asn Pro Arg Val Gln Gln

310 315 320

aaa gct caa gag gag cta gac aat gta ctt ggg tcc gaa cgt gtc ctg 1064

Lys Ala Gln Glu Glu Leu Asp Asn Val Leu Gly Ser Glu Arg Val Leu

325 330 335

acc gaa ttg gac ttc tca agc ctc cct tat cta caa tgt gta gcc aag 1112

Thr Glu Leu Asp Phe Ser Ser Leu Pro Tyr Leu Gln Cys Val Ala Lys

340 345 350 355

gag gca cta agg ctg cac cct cca aca cca cta atg ctc cct cat cgc 1160

Glu Ala Leu Arg Leu His Pro Pro Thr Pro Leu Met Leu Pro His Arg

360 365 370

gcc aat gcc aac gtc aaa att ggt ggc tac gac atc cct aag gga tca 1208

Ala Asn Ala Asn Val Lys Ile Gly Gly Tyr Asp Ile Pro Lys Gly Ser

375 380 385

aat gtt cat gta aat gtc tgg gcc gtg gct cgt gat cca gca gtg tgg 1256

Asn Val His Val Asn Val Trp Ala Val Ala Arg Asp Pro Ala Val Trp

390 395 400

cgt gac cca cta gag ttt cga ccg gaa cgg ttc tct gaa gac gat gtc 1304

Arg Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe Ser Glu Asp Asp Val

405 410 415

gac atg aaa ggt cac gat tat agg cta ctg ccg ttt ggt gca ggg agg 1352

Asp Met Lys Gly His Asp Tyr Arg Leu Leu Pro Phe Gly Ala Gly Arg

420 425 430 435

cgt gtt tgc ccc ggt gca caa ctt ggc atc aat ttg gtc aca tcc atg 1400

Arg Val Cys Pro Gly Ala Gln Leu Gly Ile Asn Leu Val Thr Ser Met

440 445 450

atg ggt cac cta ttg cac cat ttc tat tgg agc cct cct aaa ggt gta 1448

Met Gly His Leu Leu His His Phe Tyr Trp Ser Pro Pro Lys Gly Val

455 460 465

aaa cca gag gag att gac atg tca gag aat cca gga ttg gtc acc tac 1496

Lys Pro Glu Glu Ile Asp Met Ser Glu Asn Pro Gly Leu Val Thr Tyr

470 475 480

atg cga acc ccg gtg caa gct gtt ccc act cca agg ctg cct gct cac 1544

Met Arg Thr Pro Val Gln Ala Val Pro Thr Pro Arg Leu Pro Ala His

485 490 495

ttg tac aaa cgt gta gct gtg gat atg taattcttag ttgttatta 1591

Leu Tyr Lys Arg Val Ala Val Asp Met

500 505

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Pro Trp Pro Ile Val Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg Phe

35 40 45

Arg Cys Phe Ala Glu Trp Ser Gln Ala Tyr Gly Pro Ile Ile Ser Val

50 55 60

Trp Phe Gly Ser Thr Leu Asn Val Ile Val Ser Asn Ser Glu Leu Ala

65 70 75 80

Lys Glu Val Leu Lys Glu Lys Asp Gln Gln Leu Ala Asp Arg His Arg

85 90 95

Ser Arg Ser Ala Ala Lys Phe Ser Arg Asp Gly Gln Asp Leu Ile Trp

100 105 110

Ala Asp Tyr Gly Pro His Tyr Val Lys Val Thr Lys Val Cys Thr Leu

115 120 125

Glu Leu Phe Thr Pro Lys Arg Leu Glu Ala Leu Arg Pro Ile Arg Glu

130 135 140

Asp Glu Val Thr Ala Met Val Glu Ser Ile Phe Asn Asp Thr Ala Asn

145 150 155 160

Pro Glu Asn Tyr Gly Lys Ser Met Leu Val Lys Lys Tyr Leu Gly Ala

165 170 175

Val Ala Phe Asn Asn Ile Thr Arg Leu Ala Phe Gly Lys Arg Phe Val

180 185 190

Asn Ser Glu Gly Val Met Asp Glu Gln Gly Leu Glu Phe Lys Glu Ile

195 200 205

Val Ala Asn Gly Leu Lys Leu Gly Ala Ser Leu Ala Met Ala Glu His

210 215 220

Ile Pro Trp Leu Arg Trp Met Phe Pro Leu Glu Glu Gly Ala Phe Ala

225 230 235 240

Lys His Gly Ala Arg Arg Asp Arg Leu Thr Arg Ala Ile Met Glu Glu

245 250 255

His Thr Ile Ala Arg Lys Lys Ser Gly Gly Ala Gln Gln His Phe Val

260 265 270

Asp Ala Leu Leu Thr Leu Gln Glu Lys Tyr Asp Leu Ser Glu Asp Thr

275 280 285

Ile Ile Gly Leu Leu Trp Asp Met Ile Thr Ala Gly Met Asp Thr Thr

290 295 300

Ala Ile Ser Val Glu Trp Ala Met Ala Glu Leu Ile Lys Asn Pro Arg

305 310 315 320

Val Gln Gln Lys Ala Gln Glu Glu Leu Asp Asn Val Leu Gly Ser Glu

325 330 335

Arg Val Leu Thr Glu Leu Asp Phe Ser Ser Leu Pro Tyr Leu Gln Cys

340 345 350

Val Ala Lys Glu Ala Leu Arg Leu His Pro Pro Thr Pro Leu Met Leu

355 360 365
Pro His Arg Ala Asn Ala Asn Val Lys Ile Gly Gly Tyr Asp Ile Pro
370 375 380
Lys Gly Ser Asn Val His Val Asn Val Trp Ala Val Ala Arg Asp Pro
385 390 395 400
Ala Val Trp Arg Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe Ser Glu
405 410 415
Asp Asp Val Asp Met Lys Gly His Asp Tyr Arg Leu Leu Pro Phe Gly
420 425 430
Ala Gly Arg Arg Val Cys Pro Gly Ala Gln Leu Gly Ile Asn Leu Val
435 440 445
Thr Ser Met Met Gly His Leu Leu His His Phe Tyr Trp Ser Pro Pro
450 455 460
Lys Gly Val Lys Pro Glu Glu Ile Asp Met Ser Glu Asn Pro Gly Leu
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Met Asp Ser Ser Leu His Glu Ala Leu Gln Pro Leu

1 5 10

ccc atg acg ctg ttc ttc att ata cct ttg cta ctc tta ttg ggc cta 157

Pro Met Thr Leu Phe Phe Ile Ile Pro Leu Leu Leu Leu Gly Leu

15 20 25

gta tct cgg ctt cgc cag aga cta cca tac cca cca ggc cca aaa ggc 205

Val Ser Arg Leu Arg Gln Arg Leu Pro Tyr Pro Pro Gly Pro Lys Gly

30 35 40

tta ccg gtg atc gga aac atg ctc atg atg gat caa ctc act cac cga 253

Leu Pro Val Ile Gly Asn Met Leu Met Met Asp Gln Leu Thr His Arg

45 50 55 60

gga ctc gcc aaa ctc gcc aaa caa tac ggc ggt cta ttc cac ctc aag 301

Gly Leu Ala Lys Leu Ala Lys Gln Tyr Gly Gly Leu Phe His Leu Lys

65 70 75

atg gga ttc tta cac atg gtg gcc gtt tcc aca ccc gac atg gct cgc 349

Met Gly Phe Leu His Met Val Ala Val Ser Thr Pro Asp Met Ala Arg

80 85 90

caa gtc ctt caa gtc caa gac aac atc ttc tcg aac cgg cca gcc acc 397

Gln Val Leu Gln Val Gln Asp Asn Ile Phe Ser Asn Arg Pro Ala Thr

95 100 105

ata gcc atc agc tac ctc acc tat gac cga gcc gac atg gcc ttc gct 445

Ile Ala Ile Ser Tyr Leu Thr Tyr Asp Arg Ala Asp Met Ala Phe Ala

110 115 120

cac tac ggc ccg ttt tgg cgt cag atg cgt aaa ctc tgc gtc atg aaa 493

His Tyr Gly Pro Phe Trp Arg Gln Met Arg Lys Leu Cys Val Met Lys

125 130 135 140

tta ttt agc cgg aaa cga gcc gag tcg tgg gag tcg gtc cga gac gag 541

Leu Phe Ser Arg Lys Arg Ala Glu Ser Trp Glu Ser Val Arg Asp Glu

145 150 155

gtc gac tcg gca gta cga gtg gtc gcg tcc aat att ggg tcg acg gtg 589

Val Asp Ser Ala Val Arg Val Val Ala Ser Asn Ile Gly Ser Thr Val

160 165 170

aat atc ggc gag ctg gtt ttt gct ctg acg aag aat att act tac agg 637

Asn Ile Gly Glu Leu Val Phe Ala Leu Thr Lys Asn Ile Thr Tyr Arg

175 180 185

gcg gct ttt ggg acg atc tcg cat gag gac cag gac gag ttc gtg gcc 685

Ala Ala Phe Gly Thr Ile Ser His Glu Asp Gln Asp Glu Phe Val Ala

190 195 200

ata ctg caa gag ttt tcg cag ctg ttt ggt gct ttt aat ata gct gat 733

Ile Leu Gln Glu Phe Ser Gln Leu Phe Gly Ala Phe Asn Ile Ala Asp

205 210 215 220

ttt atc cct tgg ctc aaa tgg gtt cct cag ggg att aac gtc agg etc 781

Phe Ile Pro Trp Leu Lys Trp Val Pro Gln Gly Ile Asn Val Arg Leu

225 230 235
aac aag gca cga ggg gcg ctt gat ggg ttt att gac aag atc atc gac 829
Asn Lys Ala Arg Gly Ala Leu Asp Gly Phe Ile Asp Lys Ile Ile Asp
240 245 250
gat cat ata cag aag ggg agt aaa aac tcg gag gag gtt gat act gat 877
Asp His Ile Gln Lys Gly Ser Lys Asn Ser Glu Glu Val Asp Thr Asp
255 260 265
atg gta gat gat tta ctt gct ttt tac ggt gag gaa gcc aaa gta agc 925
Met Val Asp Asp Leu Leu Ala Phe Tyr Gly Glu Glu Ala Lys Val Ser
270 275 280
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Glu Ser Asp Asp Leu Gln Asn Ser Ile Lys Leu Thr Lys Asp Asn Ile
285 290 295 300
aaa gct atc atg gac gta atg ttt gga ggg acc gaa acg gtg gcg tcc 1021
Lys Ala Ile Met Asp Val Met Phe Gly Gly Thr Glu Thr Val Ala Ser
305 310 315
gcg att gaa tgg gcc atg acg gag ctg atg aaa agc cca gaa gat cta 1069
Ala Ile Glu Trp Ala Met Thr Glu Leu Met Lys Ser Pro Glu Asp Leu
320 325 330
aag aag gtc caa caa gaa ctc gcc gtg gtg gtg ggt ctt gac cgg cga 1117
Lys Lys Val Gln Gln Glu Leu Ala Val Val Val Gly Leu Asp Arg Arg
335 340 345
gtc gaa gag aaa gac ttc gag aag ctc acc tac ttg aaa tgc gta ctg 1165
Val Glu Glu Lys Asp Phe Glu Lys Leu Thr Tyr Leu Lys Cys Val Leu
350 355 360

aag gaa gtc ctt cgc ctc cac cca ccc atc cca ctc ctc ctc cac gag 1213

Lys Glu Val Leu Arg Leu His Pro Pro Ile Pro Leu Leu Leu His Glu

365 370 375 380

act gcc gag gac gcc gag gtc ggc ggc tac tac att ccg gcg aaa tcg 1261

Thr Ala Glu Asp Ala Glu Val Gly Gly Tyr Tyr Ile Pro Ala Lys Ser

385 390 395

cgg gtg atg atc aac gcg tgc gcc atc ggc cgg gac aag aac tcg tgg 1309

Arg Val Met Ile Asn Ala Cys Ala Ile Gly Arg Asp Lys Asn Ser Trp

400 405 410

gcc gac cca gat acg ttt agg ccc tcc agg ttt ctc aaa gac ggt gtg 1357

Ala Asp Pro Asp Thr Phe Arg Pro Ser Arg Phe Leu Lys Asp Gly Val

415 420 425

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Pro Asp Phe Lys Gly Asn Asn Phe Glu Phe Ile Pro Phe Gly Ser Gly

430 435 440

cgt cgg tct tgc ccc ggt atg caa ctc gga ctc tac gcg cta gag acg 1453

Arg Arg Ser Cys Pro Gly Met Gln Leu Gly Leu Tyr Ala Leu Glu Thr

445 450 455 460

act gtg gct cac ctc ctt cac tgt ttc acg tgg gag ttg ccg gac ggg 1501

Thr Val Ala His Leu Leu His Cys Phe Thr Trp Glu Leu Pro Asp Gly

465 470 475

atg aaa ccg agt gaa ctc gag atg aat gat gtg ttt gga ctc acc gcg 1549

Met Lys Pro Ser Glu Leu Glu Met Asn Asp Val Phe Gly Leu Thr Ala

480 485 490

cca aga gcg att cga ctc acc gcc gtg ccg agt cca cgc ctt ctc tgt 1597

Pro Arg Ala Ile Arg Leu Thr Ala Val Pro Ser Pro Arg Leu Leu Cys

495 500 505
cct ctc tat tgatcgaatg attgggggag ctttgtggag gggctttat 1646

Pro Leu Tyr

510
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atttccttc ctctgtggat aaaagcctcg ttttaattg ttttatgtg gagatattg 1826
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Arg Gln Arg Leu Pro Tyr Pro Pro Gly Pro Lys Gly Leu Pro Val Ile

35 40 45

Gly Asn Met Leu Met Met Asp Gln Leu Thr His Arg Gly Leu Ala Lys

50 55 60

Leu Ala Lys Gln Tyr Gly Gly Leu Phe His Leu Lys Met Gly Phe Leu

65 70 75 80

His Met Val Ala Val Ser Thr Pro Asp Met Ala Arg Gln Val Leu Gln

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115	120	125	
<u>Phe Trp Arg Gln Met Arg Lys Leu Cys Val Met Lys Leu Phe Ser Arg</u>			
130	135	140	
<u>Lys Arg Ala Glu Ser Trp Glu Ser Val Arg Asp Glu Val Asp Ser Ala</u>			
145	150	155	160
<u>Val Arg Val Val Ala Ser Asn Ile Gly Ser Thr Val Asn Ile Gly Glu</u>			
165	170	175	
<u>Leu Val Phe Ala Leu Thr Lys Asn Ile Thr Tyr Arg Ala Ala Phe Gly</u>			
180	185	190	
<u>Thr Ile Ser His Glu Asp Gln Asp Glu Phe Val Ala Ile Leu Gln Glu</u>			
195	200	205	
<u>Phe Ser Gln Leu Phe Gly Ala Phe Asn Ile Ala Asp Phe Ile Pro Trp</u>			
210	215	220	
<u>Leu Lys Trp Val Pro Gln Gly Ile Asn Val Arg Leu Asn Lys Ala Arg</u>			
225	230	235	240
<u>Gly Ala Leu Asp Gly Phe Ile Asp Lys Ile Ile Asp Asp His Ile Gln</u>			
245	250	255	
<u>Lys Gly Ser Lys Asn Ser Glu Glu Val Asp Thr Asp Met Val Asp Asp</u>			
260	265	270	
<u>Leu Leu Ala Phe Tyr Gly Glu Glu Ala Lys Val Ser Glu Ser Asp Asp</u>			
275	280	285	

Leu Gln Asn Ser Ile Lys Leu Thr Lys Asp Asn Ile Lys Ala Ile Met

290 295 300

Asp Val Met Phe Gly Gly Thr Glu Thr Val Ala Ser Ala Ile Glu Trp

305 310 315 320

Ala Met Thr Glu Leu Met Lys Ser Pro Glu Asp Leu Lys Lys Val Gln

325 330 335

Gln Glu Leu Ala Val Val Val Gly Leu Asp Arg Arg Val Glu Glu Lys

340 345 350

Asp Phe Glu Lys Leu Thr Tyr Leu Lys Cys Val Leu Lys Glu Val Leu

355 360 365

Arg Leu His Pro Pro Ile Pro Leu Leu Leu His Glu Thr Ala Glu Asp

370 375 380

Ala Glu Val Gly Gly Tyr Tyr Ile Pro Ala Lys Ser Arg Val Met Ile

385 390 395 400

Asn Ala Cys Ala Ile Gly Arg Asp Lys Asn Ser Trp Ala Asp Pro Asp

405 410 415

Thr Phe Arg Pro Ser Arg Phe Leu Lys Asp Gly Val Pro Asp Phe Lys

420 425 430

Gly Asn Asn Phe Glu Phe Ile Pro Phe Gly Ser Gly Arg Arg Ser Cys

435 440 445

Pro Gly Met Gln Leu Gly Leu Tyr Ala Leu Glu Thr Thr Val Ala His

450 455 460

Leu Leu His Cys Phe Thr Trp Glu Leu Pro Asp Gly Met Lys Pro Ser

465 470 475 480

Glu Leu Glu Met Asn Asp Val Phe Gly Leu Thr Ala Pro Arg Ala Ile

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Met Gly Ser Thr Ser Glu Thr Lys Met Ser Pro Ser Glu Ala

1	5	10
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gca gca gca gaa gaa gaa gca ttc gta ttc gct atg caa tta acc agt 156

Ala Ala Ala Glu Glu Glu Ala Phe Val Phe Ala Met Gln Leu Thr Ser

15	20	25	30
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gct tca gtt ctt ccc atg gtc cta aaa tca gcc ata gag ctc gac gtc 204

Ala Ser Val Leu Pro Met Val Leu Lys Ser Ala Ile Glu Leu Asp Val

35	40	45
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tta gaa atc atg gct aaa gct ggt cca ggt gcg cac ata tcc aca tct 252

Leu Glu Ile Met Ala Lys Ala Gly Pro Gly Ala His Ile Ser Thr Ser

50 55 60

gac ata gcc tct aag ctg ccc aca aag aat cca gat gca gcc gtc atg 300

Asp Ile Ala Ser Lys Leu Pro Thr Lys Asn Pro Asp Ala Ala Val Met

65 70 75

ctt gac cgt atg ctc cgc ctc ttg gct agc tac tct gtt cta acg tgc 348

Leu Asp Arg Met Leu Arg Leu Leu Ala Ser Tyr Ser Val Leu Thr Cys

80 85 90

tct ctc cgc acc ctc cct gac ggc aag atc gag agg ctt tac ggc ctt 396

Ser Leu Arg Thr Leu Pro Asp Gly Lys Ile Glu Arg Leu Tyr Gly Leu

95 100 105 110

gca ccc gtt tgt aaa ttc ttg acc aga aac gat gat gga gtc tcc ata 444

Ala Pro Val Cys Lys Phe Leu Thr Arg Asn Asp Asp Gly Val Ser Ile

115 120 125

gcc gct ctg tct ctc atg aat caa gac aag gtc ctc atg gag agc tgg 492

Ala Ala Leu Ser Leu Met Asn Gln Asp Lys Val Leu Met Glu Ser Trp

130 135 140

tac cac ttg acc gag gca gtt ctt gaa ggt gga att cca ttt aac aag 540

Tyr His Leu Thr Glu Ala Val Leu Glu Gly Gly Ile Pro Phe Asn Lys

145 150 155

gcc tat gga atg aca gca ttt gag tac cat ggc acc gat ccc aga ttc 588

Ala Tyr Gly Met Thr Ala Phe Glu Tyr His Gly Thr Asp Pro Arg Phe

160 165 170

aac aca gtt ttc aac aat gga atg tcc aat cat tcg acc att acc atg 636

Asn Thr Val Phe Asn Asn Gly Met Ser Asn His Ser Thr Ile Thr Met

<u>175</u>	<u>180</u>	<u>185</u>	<u>190</u>
<u>aag aaa atc ctt gag act tac aaa ggg ttc gag gga ctt gga tct gtg 684</u>			
<u>Lys Lys Ile Leu Glu Thr Tyr Lys Gly Phe Glu Gly Leu Gly Ser Val</u>			
<u>195</u>	<u>200</u>	<u>205</u>	
<u>gtt gat gtt ggt ggt ggc act ggt gcc cac ctt aac atg att atc gct 732</u>			
<u>Val Asp Val Gly Gly Gly Thr Gly Ala His Leu Asn Met Ile Ile Ala</u>			
<u>210</u>	<u>215</u>	<u>220</u>	
<u>aaa tac ccc atg atc aag ggc att aac ttc gac ttg cct cat gtt att 780</u>			
<u>Lys Tyr Pro Met Ile Lys Gly Ile Asn Phe Asp Leu Pro His Val Ile</u>			
<u>225</u>	<u>230</u>	<u>235</u>	
<u>gag gag gct ccc tcc tat cct ggt gtg gag cat gtt ggt gga gat atg 828</u>			
<u>Glu Glu Ala Pro Ser Tyr Pro Gly Val Glu His Val Gly Gly Asp Met</u>			
<u>240</u>	<u>245</u>	<u>250</u>	
<u>ttt gtt agt gtt cca aaa gga gat gcc att ttc atg aag tgg ata tgt 876</u>			
<u>Phe Val Ser Val Pro Lys Gly Asp Ala Ile Phe Met Lys Trp Ile Cys</u>			
<u>255</u>	<u>260</u>	<u>265</u>	<u>270</u>
<u>cat gat tgg agc gat gaa cac tgc ttg aag ttt ttg aag aaa tgt tat 924</u>			
<u>His Asp Trp Ser Asp Glu His Cys Leu Lys Phe Leu Lys Lys Cys Tyr</u>			
<u>275</u>	<u>280</u>	<u>285</u>	
<u>gaa gca ctt cca acc aat ggg aag gtg atc ctt gct gaa tgc atc ctc 972</u>			
<u>Glu Ala Leu Pro Thr Asn Gly Lys Val Ile Leu Ala Glu Cys Ile Leu</u>			
<u>290</u>	<u>295</u>	<u>300</u>	
<u>ccc gtg gcg cca gac gca agc ctc ccc act aag gca gtg gtc cat att 1020</u>			
<u>Pro Val Ala Pro Asp Ala Ser Leu Pro Thr Lys Ala Val Val His Ile</u>			
<u>305</u>	<u>310</u>	<u>315</u>	

gat gtc atc atg ttg gct cat aac cca ggt ggg aaa gag aga act gag 1068

Asp Val Ile Met Leu Ala His Asn Pro Gly Gly Lys Glu Arg Thr Glu

320 325 330

aag gag ttt gag gcc ttg gcc aag ggg gct gga ttt gaa ggt ttc cga 1116

Lys Glu Phe Glu Ala Leu Ala Lys Gly Ala Gly Phe Glu Gly Phe Arg

335 340 345 350

gta gta gcc tcg tgc gct tac aat aca tgg atc atc gaa ttt ttg aag 1164

Val Val Ala Ser Cys Ala Tyr Asn Thr Trp Ile Ile Glu Phe Leu Lys

355 360 365

aag att tgagtcctta ctcggctttg agtacataat accaactcct ttggttttc 1220

Lys Ile

gagattgtga ttgtgattgt gattgtctct ctttcgcagt tggccttatg atataatgta 1280

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<211> 368

<212> PRT

<213> Liquidambar styraciflua

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Ala Glu Glu Glu Ala Phe Val Phe Ala Met Gln Leu Thr Ser Ala Ser

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Val Leu Pro Met Val Leu Lys Ser Ala Ile Glu Leu Asp Val Leu Glu

35 40 45

Ile Met Ala Lys Ala Gly Pro Gly Ala His Ile Ser Thr Ser Asp Ile

50 55 60

Ala Ser Lys Leu Pro Thr Lys Asn Pro Asp Ala Ala Val Met Leu Asp

65 70 75 80

Arg Met Leu Arg Leu Leu Ala Ser Tyr Ser Val Leu Thr Cys Ser Leu

85 90 95

Arg Thr Leu Pro Asp Gly Lys Ile Glu Arg Leu Tyr Gly Leu Ala Pro

100 105 110

Val Cys Lys Phe Leu Thr Arg Asn Asp Asp Gly Val Ser Ile Ala Ala

115 120 125

Leu Ser Leu Met Asn Gln Asp Lys Val Leu Met Glu Ser Trp Tyr His

130 135 140

Leu Thr Glu Ala Val Leu Glu Gly Gly Ile Pro Phe Asn Lys Ala Tyr

145 150 155 160

Gly Met Thr Ala Phe Glu Tyr His Gly Thr Asp Pro Arg Phe Asn Thr

165 170 175

Val Phe Asn Asn Gly Met Ser Asn His Ser Thr Ile Thr Met Lys Lys

180 185 190

Ile Leu Glu Thr Tyr Lys Gly Phe Glu Gly Leu Gly Ser Val Val Asp

195 200 205

Val Gly Gly Gly Thr Gly Ala His Leu Asn Met Ile Ile Ala Lys Tyr

210 215 220

Pro Met Ile Lys Gly Ile Asn Phe Asp Leu Pro His Val Ile Glu Glu

225 230 235 240

Ala Pro Ser Tyr Pro Gly Val Glu His Val Gly Gly Asp Met Phe Val

245 250 255
Ser Val Pro Lys Gly Asp Ala Ile Phe Met Lys Trp Ile Cys His Asp
260 265 270
Trp Ser Asp Glu His Cys Leu Lys Phe Leu Lys Lys Cys Tyr Glu Ala
275 280 285
Leu Pro Thr Asn Gly Lys Val Ile Leu Ala Glu Cys Ile Leu Pro Val
290 295 300
Ala Pro Asp Ala Ser Leu Pro Thr Lys Ala Val Val His Ile Asp Val
305 310 315 320
Ile Met Leu Ala His Asn Pro Gly Gly Lys Glu Arg Thr Glu Lys Glu
325 330 335
Phe Glu Ala Leu Ala Lys Gly Ala Gly Phe Glu Gly Phe Arg Val Val
340 345 350
Ala Ser Cys Ala Tyr Asn Thr Trp Ile Ile Glu Phe Leu Lys Lys Ile
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<210> 7

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<212> DNA

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<220>

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atg gag acc caa aca aaa caa gaa gaa atc ata tat cgg tcg aaa ctc 107

Met Glu Thr Gln Thr Lys Gln Glu Glu Ile Ile Tyr Arg Ser Lys Leu

1 5 10 15

ccc gat atc tac atc ccc aaa cac ctc cct tta cat tcg tat tgt ttc 155

Pro Asp Ile Tyr Ile Pro Lys His Leu Pro Leu His Ser Tyr Cys Phe

20 25 30

gag aac atc tca cag ttc ggc tcc cgc ccc tgt ctg atc aat ggc gca 203

Glu Asn Ile Ser Gln Phe Gly Ser Arg Pro Cys Leu Ile Asn Gly Ala

35 40 45

acg ggc aag tat tac aca tat gct gag gtt gag ctc att gcg cgc aag 251

Thr Gly Lys Tyr Tyr Thr Tyr Ala Glu Val Glu Leu Ile Ala Arg Lys

50 55 60

gtc gca tcc ggc ctc aac aaa ctc ggc gtt cga caa ggt gac atc atc 299

Val Ala Ser Gly Leu Asn Lys Leu Gly Val Arg Gln Gly Asp Ile Ile

65 70 75 80

atg ctt ttg cta ccc aac tcg ccg gag ttc gtg ttt tca att ctc ggc 347

Met Leu Leu Leu Pro Asn Ser Pro Glu Phe Val Phe Ser Ile Leu Gly

85 90 95

gca tcc tac cgc ggg gct gcc gcc acc gcc gca aac ccg ttt tat acc 395

Ala Ser Tyr Arg Gly Ala Ala Ala Thr Ala Ala Asn Pro Phe Tyr Thr

100 105 110

cct gcc gag atc agg aag caa gcc aaa acc tcc aac gcc agg ctt att 443

Pro Ala Glu Ile Arg Lys Gln Ala Lys Thr Ser Asn Ala Arg Leu Ile

115	120	125	
<u>atc aca cat gcc tgt tac tat gag aaa gtg aag gac ttg gtg gaa gag 491</u>			
<u>Ile Thr His Ala Cys Tyr Tyr Glu Lys Val Lys Asp Leu Val Glu Glu</u>			
130	135	140	
<u>aac gtt gcc aag atc ata tgt ata gac tca ccc ccg gac ggt tgt ttg 539</u>			
<u>Asn Val Ala Lys Ile Ile Cys Ile Asp Ser Pro Pro Asp Gly Cys Leu</u>			
145	150	155	160
<u>cac ttc tcg gag ctg agt gag gcg gac gag aac gac atg ccc aat gta 587</u>			
<u>His Phe Ser Glu Leu Ser Glu Ala Asp Glu Asn Asp Met Pro Asn Val</u>			
165	170	175	
<u>gag att gac ccc gat gat gtg gtg gcg ctg ccg tac tcg tca ggg acg 635</u>			
<u>Glu Ile Asp Pro Asp Asp Val Val Ala Leu Pro Tyr Ser Ser Gly Thr</u>			
180	185	190	
<u>acg ggt tta cca aag ggg gtg atg cta aca cac aag gga caa gtg acg 683</u>			
<u>Thr Gly Leu Pro Lys Gly Val Met Leu Thr His Lys Gly Gln Val Thr</u>			
195	200	205	
<u>agt gtg gcg caa cag gtg gac gga gag aat ccg aac ctg tat ata cat 731</u>			
<u>Ser Val Ala Gln Gln Val Asp Gly Glu Asn Pro Asn Leu Tyr Ile His</u>			
210	215	220	
<u>agc gag gac gtg gtt ctg tgc gtg ttg cct ctg ttt cac atc tac tcg 779</u>			
<u>Ser Glu Asp Val Val Leu Cys Val Leu Pro Leu Phe His Ile Tyr Ser</u>			
225	230	235	240
<u>atg aac gtc atg ttt tgc ggg tta cga gtt ggt gcg gcg att ctg att 827</u>			
<u>Met Asn Val Met Phe Cys Gly Leu Arg Val Gly Ala Ala Ile Leu Ile</u>			
245	250	255	

atg cag aaa ttt gaa ata tat ggg ttg tta gag ctg gtc aga agt aca 875
Met Gln Lys Phe Glu Ile Tyr Gly Leu Leu Glu Leu Val Arg Ser Thr
260 265 270

ggg gac cat cat gcc tat cgt aca ccc atc gta ttg gca atc tcc aag 923
Gly Asp His His Ala Tyr Arg Thr Pro Ile Val Leu Ala Ile Ser Lys
275 280 285

act ccg gat ctt cac aac tat gat gtg tcc tcc att cgg act gtc atg 971
Thr Pro Asp Leu His Asn Tyr Asp Val Ser Ser Ile Arg Thr Val Met
290 295 300

tca ggt gcg gct cct ctg ggc aag gaa ctt gaa gat tct gtc aga gct 1019
Ser Gly Ala Ala Pro Leu Gly Lys Glu Leu Glu Asp Ser Val Arg Ala
305 310 315 320

aag ttt ccc acc gcc aaa ctt ggt cag gga tat gga atg acg gag gca 1067
Lys Phe Pro Thr Ala Lys Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala
325 330 335

ggg ccc gtg cta gcg atg tgt ttg gca ttt gcc aag gaa ggg ttt gaa 1115
Gly Pro Val Leu Ala Met Cys Leu Ala Phe Ala Lys Glu Gly Phe Glu
340 345 350

ata aaa tcg ggg gca tct gga act gtt tta agg aac gca cag atg aag 1163
Ile Lys Ser Gly Ala Ser Gly Thr Val Leu Arg Asn Ala Gln Met Lys
355 360 365

att gtg gac cct gaa acc ggt gtc act ctc cct cga aac caa ccc gga 1211
Ile Val Asp Pro Glu Thr Gly Val Thr Leu Pro Arg Asn Gln Pro Gly
370 375 380

gag att tgc att aga gga gac caa atc atg aaa ggt tat ctt aat gat 1259

Glu Ile Cys Ile Arg Gly Asp Gln Ile Met Lys Gly Tyr Leu Asn Asp

385 390 395 400

cct gag gcg acg gag aga acc ata gac aag gaa ggt tgg tta cac aca 1307

Pro Glu Ala Thr Glu Arg Thr Ile Asp Lys Glu Gly Trp Leu His Thr

 405 410 415

ggt gat gtg ggc tac atc gac gat gac act gag ctc ttc att gtt gat 1355

Gly Asp Val Gly Tyr Ile Asp Asp Asp Thr Glu Leu Phe Ile Val Asp

 420 425 430

cgg ttg aag gaa ctg atc aaa tac aaa ggg ttt cag gtg gca ccc gct 1403

Arg Leu Lys Glu Leu Ile Lys Tyr Lys Gly Phe Gln Val Ala Pro Ala

 435 440 445

gag ctt gag gcc atg ctc ctc aac cat ccc aac atc tct gat gct gcc 1451

Glu Leu Glu Ala Met Leu Leu Asn His Pro Asn Ile Ser Asp Ala Ala

 450 455 460

gtc gtc cca atg aaa gac gat gaa gct gga gag ctc cct gtg gcg ttt 1499

Val Val Pro Met Lys Asp Asp Glu Ala Gly Glu Leu Pro Val Ala Phe

465 470 475 480

ggt gta aga tca gat ggt tct cag ata tcc gag gct gaa atc agg caa 1547

Val Val Arg Ser Asp Gly Ser Gln Ile Ser Glu Ala Glu Ile Arg Gln

 485 490 495

tac atc gca aaa cag gtg gtt ttt tat aaa aga ata cat cgc gta ttt 1595

Tyr Ile Ala Lys Gln Val Val Phe Tyr Lys Arg Ile His Arg Val Phe

 500 505 510

ttc gtc gaa gcc att cct aaa gcg ccc tct ggc aaa atc ttg cgg aag 1643

Phe Val Glu Ala Ile Pro Lys Ala Pro Ser Gly Lys Ile Leu Arg Lys

515 520 525
gac ctg aga gcc aaa ttg gcg tct ggt ctt ccc aat taattctcat 1689
Asp Leu Arg Ala Lys Leu Ala Ser Gly Leu Pro Asn
530 535 540
tcgtaccct cctttctctt atcatagcc aacacgaacg aagaggctca attaaacgct 1749
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tggaagata accttcaaa aaaaaaaaaa aaaaaa 2025

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<211> 540

<212> PRT

<213> Liquidambar styraciflua

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Pro Asp Ile Tyr Ile Pro Lys His Leu Pro Leu His Ser Tyr Cys Phe

20 25 30

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35 40 45

Thr Gly Lys Tyr Tyr Thr Tyr Ala Glu Val Glu Leu Ile Ala Arg Lys

50 55 60

Val Ala Ser Gly Leu Asn Lys Leu Gly Val Arg Gln Gly Asp Ile Ile

65 70 75 80

Met Leu Leu Leu Pro Asn Ser Pro Glu Phe Val Phe Ser Ile Leu Gly

85 90 95

Ala Ser Tyr Arg Gly Ala Ala Ala Thr Ala Ala Asn Pro Phe Tyr Thr

100 105 110

Pro Ala Glu Ile Arg Lys Gln Ala Lys Thr Ser Asn Ala Arg Leu Ile

115 120 125

Ile Thr His Ala Cys Tyr Tyr Glu Lys Val Lys Asp Leu Val Glu Glu

130 135 140

Asn Val Ala Lys Ile Ile Cys Ile Asp Ser Pro Pro Asp Gly Cys Leu

145 150 155 160

His Phe Ser Glu Leu Ser Glu Ala Asp Glu Asn Asp Met Pro Asn Val

165 170 175

Glu Ile Asp Pro Asp Asp Val Val Ala Leu Pro Tyr Ser Ser Gly Thr

180 185 190

Thr Gly Leu Pro Lys Gly Val Met Leu Thr His Lys Gly Gln Val Thr

195 200 205

Ser Val Ala Gln Gln Val Asp Gly Glu Asn Pro Asn Leu Tyr Ile His

210 215 220

Ser Glu Asp Val Val Leu Cys Val Leu Pro Leu Phe His Ile Tyr Ser

225 230 235 240

Met Asn Val Met Phe Cys Gly Leu Arg Val Gly Ala Ala Ile Leu Ile

245 250 255

Met Gln Lys Phe Glu Ile Tyr Gly Leu Leu Glu Leu Val Arg Ser Thr

260 265 270

Gly Asp His His Ala Tyr Arg Thr Pro Ile Val Leu Ala Ile Ser Lys

<u>275</u>	<u>280</u>	<u>285</u>	
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<u>290</u>	<u>295</u>	<u>300</u>	
<u>Ser Gly Ala Ala Pro Leu Gly Lys Glu Leu Glu Asp Ser Val Arg Ala</u>			
<u>305</u>	<u>310</u>	<u>315</u>	<u>320</u>
<u>Lys Phe Pro Thr Ala Lys Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala</u>			
<u>325</u>	<u>330</u>	<u>335</u>	
<u>Gly Pro Val Leu Ala Met Cys Leu Ala Phe Ala Lys Glu Gly Phe Glu</u>			
<u>340</u>	<u>345</u>	<u>350</u>	
<u>Ile Lys Ser Gly Ala Ser Gly Thr Val Leu Arg Asn Ala Gln Met Lys</u>			
<u>355</u>	<u>360</u>	<u>365</u>	
<u>Ile Val Asp Pro Glu Thr Gly Val Thr Leu Pro Arg Asn Gln Pro Gly</u>			
<u>370</u>	<u>375</u>	<u>380</u>	
<u>Glu Ile Cys Ile Arg Gly Asp Gln Ile Met Lys Gly Tyr Leu Asn Asp</u>			
<u>385</u>	<u>390</u>	<u>395</u>	<u>400</u>
<u>Pro Glu Ala Thr Glu Arg Thr Ile Asp Lys Glu Gly Trp Leu His Thr</u>			
<u>405</u>	<u>410</u>	<u>415</u>	
<u>Gly Asp Val Gly Tyr Ile Asp Asp Asp Thr Glu Leu Phe Ile Val Asp</u>			
<u>420</u>	<u>425</u>	<u>430</u>	
<u>Arg Leu Lys Glu Leu Ile Lys Tyr Lys Gly Phe Gln Val Ala Pro Ala</u>			
<u>435</u>	<u>440</u>	<u>445</u>	
<u>Glu Leu Glu Ala Met Leu Leu Asn His Pro Asn Ile Ser Asp Ala Ala</u>			
<u>450</u>	<u>455</u>	<u>460</u>	
<u>Val Val Pro Met Lys Asp Asp Glu Ala Gly Glu Leu Pro Val Ala Phe</u>			
<u>465</u>	<u>470</u>	<u>475</u>	<u>480</u>

Val Val Arg Ser Asp Gly Ser Gln Ile Ser Glu Ala Glu Ile Arg Gln

485 490 495

Tyr Ile Ala Lys Gln Val Val Phe Tyr Lys Arg Ile His Arg Val Phe

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Phe Val Glu Ala Ile Pro Lys Ala Pro Ser Gly Lys Ile Leu Arg Lys

515 520 525

Asp Leu Arg Ala Lys Leu Ala Ser Gly Leu Pro Asn

530 535 540

<210> 9

<211> 1544

<212> DNA

<213> Pinus taeda

<400> 9

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atattagtga aagctagtta aactaacccc ttgacttgc aagatgatatt attatatcc 180

ctactacgtc ttctctttt tgtcttctc ttgtgattaa accttcttg aaacaattct 240

caaatgtaaa attaaacctt gaaacttgta gagaccaaac ttcctagga gaaaccacat 300

ttatgacaac atatatcac caaccattg catactataa tattggaatt acctgcagcg 360

aacgaaagaa acgctgtctc accaactcgt gcactacatc ccgaaactta accttccct 420

gatacagatt gaagagccga aaaaagcgtg catccaaatt tctggtatgg tgaggagccg 480

aaaaacgcgt ggcctaatt ttttgagat gggccggaaa ataatgcgtg catctaaatt 540

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<210> 10

<211> 659

<212> DNA

<213> Pinus taeda

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cggaaaacag cgaatgaaat gtctgggtga tcggtcaaac aagcgggtgg cgagagagcg 180

cggtgttg cctagccggg atgggggtag gtagacggcg tattaccggc gagttgtccg 240
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caaaaatcca accgctcctt cacatcgag agttggtggc cacgggaccc tccaccact 360
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accaacaatt ccaggccggc ttctataca atgtactgca caggaaaatc caatataaaa 480
agccggcctc tgettcttc tcagtagccc ccagctcatt caattcttc cactgcaggc 540
tacattgtc agacacgtt tccgccattt ttcgctgtt tctgcggaga atttgatcag 600
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<210> 11

<211> 2251

<212> DNA

<213> Pinus taeda

<400> 11

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aaaccttaa tataaagaat tctttcaac aatacactt aatcacaact tcttcaatca 180
caacctctc caacaaaatt aaaatagatt aataaataa taaacttaac tatttaaaaa 240
aaaatattat acaaaattta ttaaaacttc aaaataaaca aactttttat acaaaattca 300
tcaaaacttt aaaataaagc taaacactga aaatgtgagt acatttaaaa ggacgctgat 360
cacaaaaatt tgaaaaacat aaacaaactt gaaactctac ctttaagaa tgagttgtc 420
gttcattaa ctcatagtt ttatagttcg aatccaatta acgtatctt tattttatgg 480
aataagggtg tttaataag tgatttggg atttttttag taatttatt gtgatatgtt 540
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tggaaaagggt tgtaagaac tataaattga gttgtgaatg agtgttttat ggattttta 660
agatgttaaa ttatatatg taattaaaat ttattttga ataacaaaa ttataattgg 720

ataaaaaatt gttttgttaa atttagagta aaaatttcaa aatctaaaat aattaaacac 780
tattattttt aaaaaatttg ttggtaaatt ttatcttata ttttaagttaa aatttagaaa 840
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caatttgat aaaaacaaaa aatctcaaat aaaatttaaa ttacaaaaca ttatcaacat 1020
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26

What is claimed is:

1. A method for modifying the genome of a gymnosperm which comprises cloning one or more angiosperm DNA sequences which code for genes necessary for production of angiosperm syringyl lignin monomer units, fusing one or more of the angiosperm DNA sequences to a promoter region associated with a gene to form an expression cassette and inserting the expression cassette into the gymnosperm to thereby produce a modified genome in the gymnosperm containing genes which code for enzymes which produce syringyl lignin monomer units.
2. The method of claim 1, further comprising incorporating a genetic sequence which codes for anti-sense mRNA into the gymnosperm in order to suppress formation of ; guaiacyl lignin monomer units.
3. A gymnosperms plant containing an expression cassette produced according to the method of claim 1.
4. A loblolly pine containing an expression cassette produced according to the method of claim 1.
5. The method of claim 1 wherein the angiosperm DNA sequences are selected from the class consisting of 4-coumarate CoA ligase (4CL), bifunctional-O-methyl transferase (bi-OMT) and ferulic acid-5-hydroxylase (FA5H-1 and FA5H-2).
6. The method of claim 1 wherein the promoter region is selected from the class consisting of the 5' flanking region of phenylalanine ammonia-lyase (PAL) and the 5' flanking region of 4-coumarate CoA ligase (4CL1B and 4CL3B).
7. The method of claim 1 wherein the expression cassette is inserted into the gymnosperm genome by way of the transformation vector *Agrobacterium*.
8. The method of claim 7 wherein the *Agrobacterium* is *Agrobacterium tumefaciens* EH101.

9. The method of claim 1 wherein the expression cassette is inserted into the gymnosperm genome via direct DNA delivery to a target cell.
10. The method of claim 1 wherein expression cassette is inserted into the gymnosperm genome by micro-projectile bombardment of a gymnosperm cell.
11. The method of claim 1 wherein the expression cassette is inserted into the gymnosperm genome by electroporation of a gymnosperm cell.
12. The method of claim 1 wherein the expression cassette is inserted into the gymnosperm genome via silicon carbide whiskers.
13. The method of claim 1 wherein the expression cassette is inserted into the gymnosperm genome via transformed protoplast.
14. The method of claim 1 further comprising inserting a selectable marker into the expression cassette.
15. The method of claim 14 wherein the selectable marker is selected from the group consisting of kanamycin and hygromycin B.
16. The method of claim 2 wherein the anti-sense mRNA is a gymnosperm genetic sequence which codes for the 4-coumarate CoA ligase (4CL) gene.
17. The method of claim 1 wherein the promoter region is a DNA sequence which includes the 5' flanking region of the gymnosperm loblolly pine PAL gene.
18. The method of claim 1 wherein the promoter, region is a DNA sequence which includes the 5' flanking region of the gymnosperm loblolly pine 4CL1B gene.
19. The method of claim 1 wherein the promoter, region is a DNA sequence which includes the 5' flanking region of the gymnosperm loblolly pine 4CL3B gene.
20. The method of claim 1 wherein the promoter region includes a constitutive promoter.

21. An isolated FA5H-1 DNA sequence which encodes an enzyme involved in the biosynthesis of syringyl lignin monomer units, wherein said DNA is as shown in SEQ ID. No. 1.

22. An isolated FA5H-2 DNA sequence which encodes an enzyme involved in the biosynthesis of syringyl lignin monomer units, wherein said DNA is as shown in SEQ ID. No. 2.

23. An isolated bi-OMT DNA sequence which encodes an enzyme involved in the biosynthesis of syringyl lignin monomer units, wherein said DNA is as shown in SEQ ID. No. 3.

24. An isolated 4CL DNA, sequence which encodes an enzyme involved in the biosynthesis of syringyl lignin monomer units, wherein said DNA is as shown in SEQ ID. No. 4.

25. An isolated DNA, wherein said DNA encodes for an enzyme involved in the biosynthesis one or more syringyl lignin monomer units.

26. An isolated DNA sequence which includes the 5' flanking region of the gymnosperm loblolly pine PAL gene, containing the lignin promoter region and regulatory elements for gymnosperm lignin biosynthesis as shown in SEQ ID No. 5.

27. An isolated DNA sequence which includes the 5' flanking region of the gymnosperm loblolly pine 4C1B, containing the lignin promoter region and regulatory elements for gymnosperm lignin biosynthesis as shown in SEQ ID No. 6.

28. An isolated DNA sequence which includes the 5' flanking region of gymnosperm loblolly pine 4CL3H, containing the lignin promoter region and regulatory elements for gymnosperm lignin biosynthesis as shown in SEQ ID No. 7.

29. An isolated DNA, wherein said DNA includes the promoter region of a gymnosperm gene involved in syringyl lignin biosynthesis.

30. A method for modifying the genome of loblolly pine which comprises cloning one or more angiosperm DNA sequences which code for enzymes necessary for production of syringyl lignin monomer units, fusing one or more of the angiosperm DNA sequences to a promoter region to form an expression cassette, and inserting the expression cassette into the loblolly pine genome to thereby produce a modified genome in the loblolly pine containing genes which code for enzymes which produce syringyl lignin monomer units.

31. The method of claim 30 wherein the promoter region is a constitutive promoter.

32. A loblolly pine containing an expression cassette produced according to claim 30.

33. The method of claim 30 wherein the angiosperm DNA sequence is selected from the class consisting of 4-coumarate CoA ligase (4CL), bifunctional-O-methyl transferase (bi-OMT) and ferulic acid-5-hydroxylase (FA5H-1 and FA5H-9)

34. A loblolly pine containing one or more of the DNA sequences of claim 33.

35. A loblolly pine containing the angiosperm DNA sequence inserted by the method of claim 30.

36. A method for modifying the genome of loblolly pine which comprises cloning the sweetgum FA5H-1 gene, fusing it to a constitutive promoter to form an expression cassette, and inserting the expression cassette into the loblolly pine genome.

37. A loblolly pine containing the FA5H-1 gene.

38. A method for modifying the genome of loblolly pine which comprises cloning the sweetgum FA5H-2 gene, fusing it to a constitutive promoter to form an expression cassette, and inserting the expression cassette into the loblolly pine genome.

39. A loblolly pine containing the FA5H-2 gene.

40. A method for modifying the genome of a gymnosperm which comprises cloning the sweetgum FA5H-1 gene, fusing it to a constitutive promoter to form an expression cassette, and inserting the expression cassette into the loblolly pine genome.

41. A method for modifying the genome of a gymnosperm which comprises cloning the sweetgum FA5H-2 gene, fusing it to a constitutive promoter to form an expression cassette, and inserting the expression cassette into the loblolly pine genome.

42. A gymnosperm containing the FA5H-1 gene.

43. A gymnosperm containing the FA5H-2 gene.

44. A gymnosperm containing a DNA sequence selected from the class consisting of the FA5H-1 DNA sequence of SEQ ID No. 1, the FA5H-2 DNA sequence of SEQ ID No. 2, the OMT DNA sequence of SEQ ID No. 3, and the 4CL DNA sequences of SEQ ID No. 4.

45. The gymnosperm of claim 38, further comprising syringyl lignin.

PRODUCTION OF SYRINGYL LIGNIN

Abstract

The present invention relates to a method for producing syringyl lignin in gymnosperms. The production of syringyl lignin in gymnosperms is accomplished by genetically transforming a gymnosperm genome, which does not normally contain genes which code for enzymes necessary for production of syringyl lignin, with DNA which codes for enzymes found in angiosperms associated with production of syringyl lignin. The expression of the inserted DNA is mediated using host promoter regions in the gymnosperm. In addition, genetic sequences which code for gymnosperm lignin anti-sense mRNA may be incorporated into the gymnosperm genome in order to suppress the formation of the less preferred forms of lignin in the gymnosperm such as guaiacyl lignin